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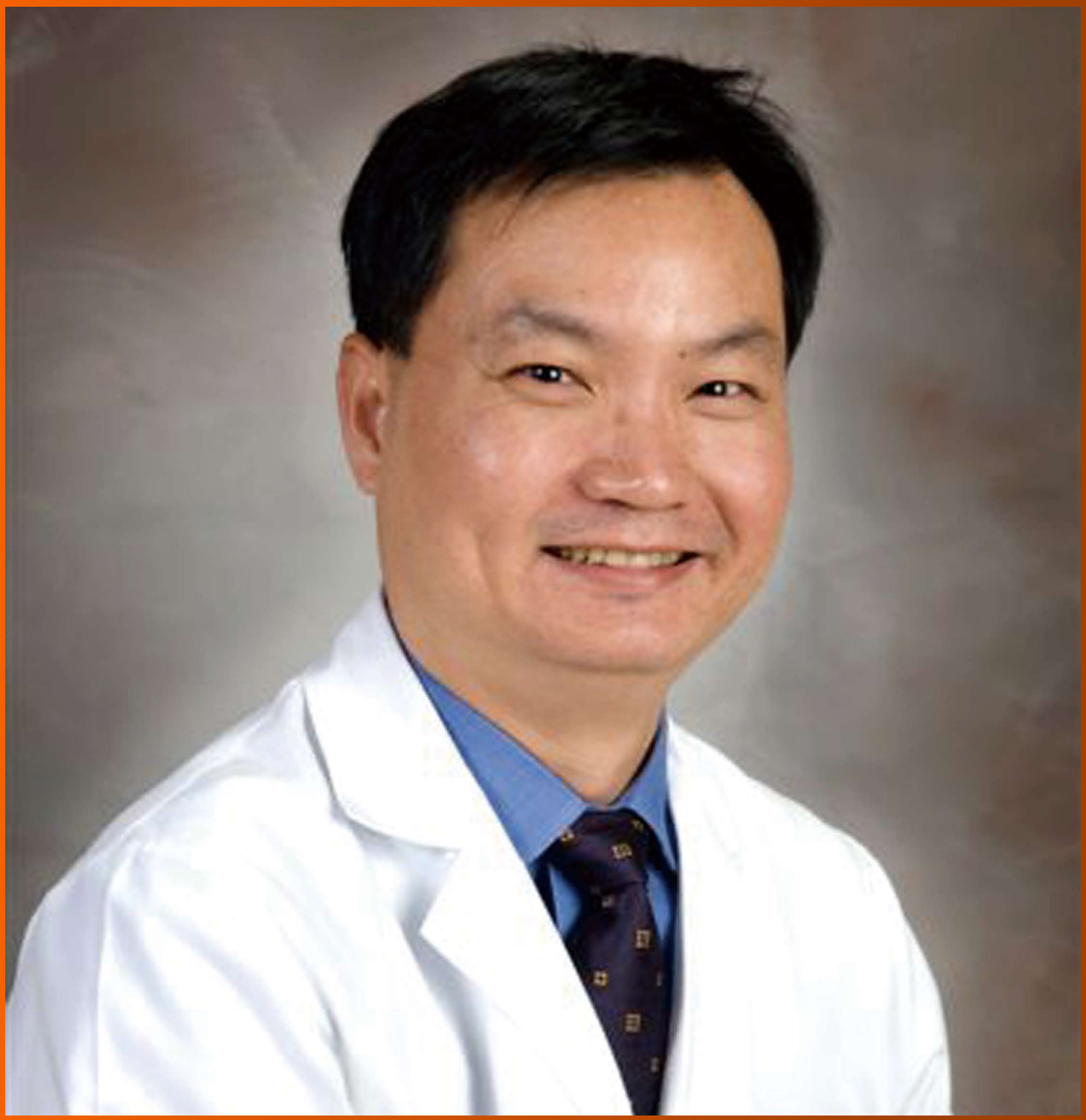

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EDITORIAL

Understanding the pathophysiological mechanisms in the pediatric non-alcoholic fatty liver disease: The role of genetics

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Abstract

Classically, the non-alcoholic fatty liver disease (NAFLD) physiopathology and progression has been summarized in the two hits hypothesis. The first hit is represented by the action of hyperinsulinemia and insulin resistance, accompanying obesity, that leads to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol. The oxidative stress is involved in the second hit leading to the progression to nonalcoholic steatohepatitis (NASH) because of its harmful action on steatosic hepatocytes. However, at the present time, the two hits hypothesis needs to be updated because of the discover of genetic polymorphisms involved both in the liver fat accumulation and progression to NASH that make more intriguing understanding the NAFLD pathophysiological mechanisms. In this editorial, we want to underline the role of *PNPLA3* I148M, *GPR120* R270H and *TM6SF2* E167K in the pediatric NAFLD development because they add new pieces to the comprehension of the NAFLD pathophysiological puzzle. The *PNPLA3* I148M polymorphism encodes for an abnormal protein which predisposes to intrahepatic triglycerides accumulation both for a loss-of-function of its triglyceride hydrolase activity and for a gain-of-function of its lipogenic activity. Therefore, it is involved in the first hit, such as *TM6SF2* E167K polymorphisms that lead to intrahepatic fat accumulation through a reduced very low density lipoprotein secretion. On the other hand, the *GPR120* R270H variant, reducing the anti-inflammatory action of the GPR120 receptor expressed by Kuppfer cells, is involved in the second hit leading to the liver injury.

Key words: Pediatric non-alcoholic fatty liver disease; *GPR120*; *PNPLA3*; *TM6SF2*; Alanine transaminase

Core tip: At the present time, the two hits hypothesis needs to be updated because of the discovery of new genetic polymorphisms involved both in the liver fat accumulation and progression to nonalcoholic steatohepatitis that make more intriguing understanding

the non-alcoholic fatty liver disease (NAFLD) pathophysiological mechanisms. In this editorial, that is not to consider as a comprehensive review, we want to underline the role of three polymorphisms, one older (*PNPLA3* I148M) but very important and two recently discovered (*GPR120* R270H and *TM6SF2* E167K) that add new pieces to the comprehension of the NAFLD pathophysiological puzzle.

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INTRODUCTION

In the last years, the pediatric obesity prevalence has shown a constant increase[1]. The raised pediatric obesity prevalence has determined an increased prevalence of obesity complications and the non-alcoholic fatty liver disease (NAFLD) has become the most common form of liver disease in childhood. In fact, its prevalence has more than doubled over the past years. It currently involves between 3% and 11% of the pediatric population and affects about the 46% of overweight and obese children and adolescents[2]. NAFLD comprehends an extensive range of conditions, including from fatty liver or steatohepatitis with or without fibrosis, to cirrhosis and its complications (*e.g.*, hepatocellular carcinoma and portal hypertension)[3,4]. The NAFLD is defined by hepatic fat infiltration involving > 5% hepatocytes in the absence of excessive alcohol intake or other demonstrable liver diseases[3].

Classically, the NAFLD pathophysiology and progression has been summarized in the two hits hypothesis. Obesity related hyperinsulinemia and insulin resistance represent the first hit. They lead to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol[5,6]. The oxidative stress is involved in the second hit leading to the progression to NASH because of its harmful action on steatosic hepatocytes. The reactive oxygen species (ROS), in fact, lead to hepatocellular damage inhibiting the mitochondrial respiratory chain enzymes, and inactivating both the glyceraldehyde-3-phosphate dehydrogenase and the membrane sodium channels. ROS further cause lipid peroxidation, cytokine production, and induce Fas Ligand, contributing to hepatocellular injury and fibrosis[7].

However, at the present time, the two hits hypothesis needs to be updated because of the discover of 3 genes whose polymorphisms are involved both in the liver fat accumulation and progression to non-alcoholic steatohepatitis (NASH) making intriguing understanding the NAFLD pathophysiological mechanisms. In this editorial, that is not to consider as a comprehensive review, we want to underline the role of three polymorphisms, one older but very important and two recently discovered that added new pieces to the comprehension of the NAFLD pathophysiological puzzle.

PATATIN LIKE PHOSPHOLIPASE CONTAINING DOMAIN 3 GENE

The patatin like phospholipase containing domain 3 gene (*PNPLA3*) is the most important gene involved in hepatic steatosis developing. It encodes for the adiponutrin, an enzyme present in the liver and adipose tissue. Feeding and insulin resistance induce the adiponutrin[8] that shows lipolytic activity on triglycerides[9]. The *PNPLA3* rs738409 (*PNPLA3* I148M) single nucleotide polymorphism is a non-synonymous variant and it is characterized by a cytosine to guanosine substitution leading to an isoleucine to methionine substitution at the amino acid position 148 (I148M). This aminoacid substitution affects the enzyme function probably reducing the substrates access to the enzyme and then leading to the development of microvesicular steatosis[9]. On the other hand, the adiponutrin could present a gain of lipogenic function, which could further lead to the hepatic fatty acids accumulation[9]. In literature there is strong evidence of association between the *PNPLA3* 148M allele and NAFLD both in adults[10] and children[11,12].

The *PNPLA3* 148M allele plays a central role in NAFLD developing interacting with environmental NAFLD risk factors, such as obesity (and in particular visceral fat)[11] and alcohol consumption[13], and then increasing the risk of fatty liver development. In fact, these stressors seem to reveal the association between the *PNPLA3* 148M allele and hepatic damage in populations in whom it is otherwise hidden[14]. Interestingly, the obesity-driven effect of this polymorphism on liver damage[11] can be reduced by weight loss (expressed as reduction of the waist/height ratio) in obese children and adolescents[15]. Among environmental factors involved in NAFLD development and interacting with *PNPLA3* 148M allele some nutrients appear. Indeed, the total carbohydrate[16] and high omega (n) 6 to n-3 polyunsaturated fatty acids (PUFA) ratio[17] can enhance the association between steatosis and the *PNPLA3* variant.

G-PROTEIN-COUPLED-RECEPTOR 120

G-protein-coupled-receptor 120 *(GPR120)* is a receptor for PUFAs and it is expressed by adipocytes, Kupffer cells and, at low level, in hepatocytes[18]. PUFAs could play a role in inflammatory response modulation[19]. In fact, it has been shown that, in the adipose tissue, the interaction between PUFAs and macrophagic *GPR120* switch off inflammation blocking nuclear factor-kappa-B activity[20]. In particular docosahexaenoic acid (DHA), an n-3 PUFA of the fish oil, has recently shown a potential

role in treatment of liver fat accumulation and of metabolic and hepatic complications of NAFLD[21]. The 270H allele inhibits the *GPR120* signalling activity[19] and then it reduce its anti-inflammatory activity[20]. Recently, this variant has been studied in obese children and adolescents and it has been shown that the subject carrying the rare *GPR120* 270H allele presented higher alanine transaminase (ALT) levels ($P = 0.01$) and higher ferritin (marker of inflammation) levels ($P < 0.003$) than wild type subjects[22]. The carriers of the 270H allele showed an odds ratio (OR) to have pathologic ALT of 3.2[22]. Moreover, *PNPLA3* 148M allele demonstrated an interaction with *GPR120* 270H allele determining a significant effect on ALT levels ($P = 0.00001$) suggesting a driving effect of the *PNPLA3* 148M allele on liver injury in obese children carrying this variant[22]. This is in accord with the findings of Santoro *et al*[17] demonstrating that the *PNPLA3* I148M variant predispose to liver damage in patients with a lower intake of n-3 fatty acids. Therefore, GPR120 R270H variant appear to have an important role in determining liver injury expressed as ALT elevation and the *PNPLA3* 148M allele showed an important capacity to promote the GPR120 270H allele mediated liver damage. This evidence is in accord with the studies showing that DHA supplementation, activating the GPR120 receptor and then exerting potent anti-inflammatory and insulin-sensitizing activities[20], can reduced liver damage in pediatric NAFLD[23].

TRANSMEMBRANE 6 SUPERFAMILY MEMBER 2 GENE

Recently, a new gene variant playing a role in the NAFLD physiopathology has been discovered in the transmembrane 6 superfamily member 2 (*TM6SF2*) gene[24]. This variant (rs58542926) is characterized by an adenine-to-guanine substitution in the nucleotide 499 which replaces glutamate at residue 167 with lysine (c.499A > G; p.Glu167Lys)[24]. The *TM6SF2* minor allele carriage has been causally related to a previously reported chromosome 19 GWAS signal that was ascribed to the gene *NCAN*[25]. This *TM6SF2* variant has been associated with higher hepatic triglyceride content (HTGC), with higher serum levels of ALT and lower plasma levels of liver-derived triglyceride-rich lipoproteins in 3 independent populations[24].

Small intestine, liver and kidney highly express the *TM6SF2* gene, but, in the other tissues, *TM6SF2* is present at low levels[24]. Recent evidence suggests that TM6SF2 is a polytopic membrane protein and that the Glu167Lys variant form is misfolded and undergoes accelerated intracellular degradation[24]. Actually, the hypothesized TM6SF2 protein function appears to be the promotion of very low density lipoprotein (VLDL) secretion and, probably, the increased HTGC result from a reduction in TM6SF2 protein function[24,26]. Therefore, the role of the *TM6SF2* 167K allele in the NAFLD physiopathology could be represented by a reduced VLDL secretion that could explain the higher HTGC, in turn resulting in higher ALT levels and in lower serum low density lipoprotein cholesterol (LDL-C) and triglycerides levels. In addition, low cholesterol levels in the carrier of the *TM6SF2* 167K allele have also been demonstrated in an adult population presenting, at the same time, reduced risk of myocardial infarction[27]. Accordingly, very recent data showed an effect of this polymorphism on reducing carotid atherosclerosis risk in adults[28]. Moreover, Liu *et al*[25] confirmed, using two histologically characterized cohorts (1074 adults) encompassing steatosis, steatohepatitis, fibrosis and cirrhosis, the *TM6SF2* minor allele association with NAFLD and, moreover, with advanced hepatic fibrosis/cirrhosis. The effect of this polymorphism on ALT and cholesterol levels has been confirmed also in obese children and adolescents[29]. Grandone *et al*[29], in fact, demonstrated, in a cohort of 1010 obese children and adolescents, that the *TM6SF2* 167K allele is associated with steatosis ($P < 0.0001$), higher ALT levels ($P < 0.001$) and lower total cholesterol (< 0.00001), LDL-C ($P < 0.0001$), triglycerides ($P = 0.02$) and non-high density lipoprotein cholesterol levels ($P < 0.000001$)[29]. Interestingly, the subjects homozygous for the *PNPLA3* 148M allele carrying the rare variant of *TM6SF2* showed an OR of 12.2 to present hypertransaminasemia compared to the remaining patients[29]. Therefore, the effect of *PNPLA3* and *TM6SF2* rare alleles appears additive in determining pediatric NAFLD[29].

OLD AND NEW CONCEPTS, AN INTEGRATED OVERVIEW

NAFLD occurs in overweight and obese children deriving from intrahepatic accumulation of triglycerides. The triglycerides accumulated in the liver mostly derive from the adipose tissue lipolysis (60%) and hepatic *de novo* lipogenesis (26%) whereas only in a little part from the diet as chylomicron remnants (14%)[30]. This fat accumulation, as indicated in the "two hits" hypothesis (Figure 1), is stimulated by obesity-related peripheral insulin resistance and hyperinsulinemia. In fact they stimulate the free-fatty-acids (FFA) uptake in the liver, the esterification of hepatic FFAs to form triglycerides, the FFA synthesis from cytosolic substrates, and the decreased apolipoprotein B-100 synthesis. Then, the export of FFA and triglycerides decreases, while the beta-oxidation of mitochondrial long-chain fatty acids increases[2]. In the last years, the knowledge of NAFLD pathophysiology is constantly increasing[8] with the discovery of new genetic polymorphisms that could promote the NAFLD development and then the progression to the NASH. Each polymorphism plays a role in a different hit and, therefore, in future, the pathophysiology could be described by a "multiple hit hypothesis". *PNPLA3* I148M polymorphism plays an important role in the NAFLD development. In fact, it

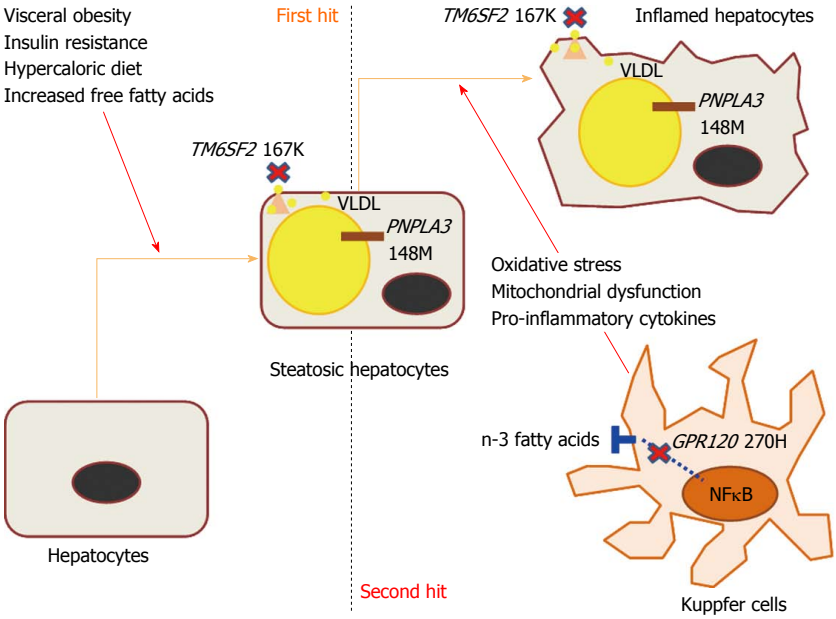

Figure 1 The updated "two hits hypothesis". The hyperinsulinemia and insulin resistance, accompanying obesity, lead to liver steatosis increasing the absolute non esterified fatty acids uptake in the liver and the esterification to form triacylglycerol. The *PNPLA3* 148M allele encodes for an abnormal protein and predisposes to intrahepatic triglycerides accumulation by both a reduced effect on triglycerides hydrolysis and an enhanced lipogenetic effect. Therefore, it is involved in the first hit. Also the *TM6SF2* E167K polymorphism plays a role in the first hit, in fact, it lead to intrahepatic fat accumulation through a reduced VLDL secretion. The oxidative stress is involved in the second hit leading to the progression to NASH because of its harmful action on steatosic hepatocytes. Reactive oxygen species can induce hepatocellular injury and then fibrosis through the inhibition of the mitochondrial respiratory chain enzymes, lipid peroxidation, cytokine production, Fas Ligand induction. The *GPR120* 270H allele, reducing the anti-inflammatory action of the GPR120 receptor expressed by Kuppfer cells, is involved in the second hit promoting the oxidative stress, mitochondrial dysfunction and pro-inflammatory cytokines release. *PNPLA3*: Patatin like phospholipase containing domain 3 gene; NASH: Nonalcoholic steatohepatitis; *GPR120*: G-protein-coupled-receptor 120; *TM6SF2*: Transmembrane 6 superfamily member 2 gene; NFκB: Nuclear factor-kappa-B; VLDL: Very low density lipoprotein.

encodes for an abnormal protein which predisposes to intrahepatic triglycerides accumulation[9,12] both for a loss-of-function of its triglyceride hydrolase activity and for a gain-of-function of its lipogenic activity[12]. Therefore, the *PNPLA3* I148M polymorphism plays a role in predisposing to the first hit (Figure 1). Another polymorphism acting on the first hit is the *TM6SF2* E167K polymorphism. In fact, it encodes for an abnormal TM6SF2 protein that predisposes to intrahepatic fat accumulation thorough reduced VLDL secretion[24,26] (Figure 1). On the other hand, the reduced VLDL secretion lead to a reduced cardiovascular risk[27,28]. The *GPR120* R270H variant promotes the second hit determining liver injury (evaluable as high ALT levels)[22]. In fact, the lack of *GPR120* anti-inflammatory activity promotes oxidative stress, mitochondrial dysfunction, overproduction and release of pro-inflammatory cytokines (second hit) (Figure 1). This hypothesis appears further supported by the evidence that the *GPR120* mutated allele needs the co-presence of the *PNPLA3* 148M allele to lead pathological ALT levels[22].

Therefore, the *PNPLA3* 148M allele can play an important role in the first hit determining hepatic steatosis and can drive the effect of the *GPR120* rare allele on the second hit leading to liver damage.

CONCLUSION

In the last years many polymorphisms playing a role in the NAFLD physiopathology have been identified[8], therefore our knowledge in this area has been constantly increased. Starting from the genetic association studies we can understand new pathophysiological mechanisms that are firstly implicated in the intrahepatic fat accumulation and then, in the progression to the NASH (Figure 1). This editorial wants to underline how these new genetic findings have improved our comprehension of pediatric NAFLD pathophysiology.

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EDITORIAL

Liver-first approach of colorectal cancer with synchronous hepatic metastases: A reverse strategy

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Abstract

Recently, there has been a change in the strategy of how synchronous colorectal hepatic metastases are attributed to the development of more valuable protocols of chemotherapy and radiotherapy for neoadjuvant treatment of colorectal neoplasms and their hepatic metastases. There is a consensus that patients with synchronous colorectal hepatic metastases have lower survival than those with metachronous colorectal hepatic metastases. Currently, controversy remains concerning the best approach is sequence in a patient with colorectal cancer and synchronous hepatic metastases resection. To obtain a better patient selection, the authors have suggested the initial realization of systemic chemotherapy in the circumstance of patients with colorectal tumor stage Ⅳ, since these patients have a systemic disease. The rationale behind this liver-first strategy is initially the control of synchronous hepatic metastases of colorectal carcinoma, which can optimize a potentially curative hepatic resection and longstanding survival. The liver-first strategy procedure is indicated for patients with colorectal hepatic metastases who require downstaging therapy to make a curative liver resection possible. Thus, the liver-first strategy is considered an option in cases of rectal carcinoma in the early stage and with limited or advanced synchronous colorectal hepatic metastases or in case of patients with asymptomatic colorectal carcinoma, but with extensive liver metastases. Patients undergoing systemic chemotherapy and with progression of neoplastic disease should not undergo hepatic resection, because it does not change the prognosis and may even make it worse. To date, there have been no randomized controlled trials on surgical approach of colorectal synchronous hepatic metastases, despite the relatively high number of available manuscripts on this subject. All of these published studies are observational, usually retrospective, and often non-comparative. The patient selection criteria for the liver-first strategy should be individualized, and the approach of these patients should be performed by a multidisciplinary team so its benefits will be fully realized.

Key words: Colorectal neoplasms; Neoplasm metastasis;

Liver neoplasms; Liver/surgery; Hepatectomy; Drug therapy; Survival; Prognosis

Core tip: The liver-first approach or reverse strategy is a downstaging regimen, and it consists of systemic chemotherapy, chemoradiotherapy and/or biological agents, followed by resection of colorectal hepatic metastases prior to removal the primary colorectal tumor. It is a promising strategy in patients with synchronous colorectal liver metastases. The rationale behind this liver-first strategy is initially control of synchronous hepatic metastases of colorectal carcinoma, which can optimize the opportunity of a potentially curative liver resection and longstanding survival. The liver-first strategy can be applied for patients with early stage colorectal carcinoma and synchronous hepatic metastases. Extensive or locally advanced rectal carcinoma with limited or advanced synchronous hepatic metastases and asymptomatic colonic carcinoma with extensive synchronous hepatic metastases may be submitted to the liver-first strategy.

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TEXT

Colorectal cancer remains the fourth most common malignancy in the United States, being the third most common cancer in both men and women[1]. Approximately 15% to 25% of these patients present with colorectal synchronous hepatic metastases detected either before or during operation[2]. There is a consensus that patients with synchronous colorectal hepatic metastases have lower survival than those with hepatic colorectal metachronous metastases[3], and so this finding is considered a poor prognosis predictor[4]. However, the presence of synchronous colorectal hepatic metastases does not exclude the potential of long-standing survival and the opportunity of cure[5].

It is agreed in the medical literature that surgical resection is considered the only curative option for patients with colorectal carcinoma and synchronous hepatic metastases[6]. Currently, combinations of three treatment regimens have been implemented in the treatment of colorectal synchronous hepatic metastases: preliminary resection of the colorectal tumor; concurrent resection of colorectal tumor and synchronous hepatic metastases; and the liver-first strategy, wherein resection of synchronous hepatic colorectal metastases precedes the resection of the primary colorectal tumor[5,6].

The conventional surgical strategy for patients with resectable synchronous colorectal hepatic metastases includes resection of the colorectal carcinoma, followed by chemotherapy, and eventually synchronous hepatic metastases resection[6]. The basis for this traditional approach is that the colorectal tumor is considered the common cause of symptoms and metastases[6,7].

There are the following arguments in favor of an initial colorectal cancer resection: in patients with complications due to tumor (obstruction, bleeding, or perforation) and who require emergency surgery to control these complications, the interval time period between the colorectal resection and hepatic resection of synchronous metastases may exclude liver resection because in patients undergoing systematic chemotherapy, occult metastatic disease may become detectable[7,8]. Furthermore, in patients with symptomatic colorectal carcinoma, the liver-first strategy is not suitable[2].

Consequently, patients with disease progression and unresectable disease should not undergo hepatic resection, avoiding the perioperative morbidity and mortality of hepatic surgery without benefit to the patient[8]. Furthermore, in patients with a locally advanced primary tumor there is a risk of complication while on chemotherapy[9]. So the preferred alternative is surgery of the colorectal tumor first to avoid complications related to this tumor and the need for emergency surgery with a high risk of stoma creation[9].

On the other hand, there are arguments for not performing a preliminary colorectal resection in patients with colorectal carcinoma and synchronous colorectal hepatic metastases: the response of preoperative chemotherapy with oxaliplatin-and/or irinotecan-based regimens in colorectal cancer is correlated with a significantly corresponding response of colorectal hepatic metastases.

However, colorectal cancer resection in patients with hepatic metastases was related with a significantly higher postoperative mortality when compared with colorectal cancer resection in patients without liver metastases; the complications of leaving intact the colorectal cancer are not as high and can be overestimated; disease progression between the timing of the colorectal and hepatic surgeries may render the colorectal hepatic metastases unresectable, particularly when there are postoperative complications after colorectal cancer resection, preventing systemic chemotherapy and resection of colorectal hepatic metastases; and the main determinant of the patient's survival is the presence of systemic metastases, and the treatment of colorectal hepatic metastases should be the initial priority[7].

In some cases, in particular with patients with colonic carcinoma, the approach should be neo-adjuvant therapy following the simultaneous resection approach. The traditional approach should be considered in patients with limited synchronous disease who do not require downstaging.

In the synchronous strategy, the hepatic metastases and colorectal tumor are resected simultaneously[6] with

the benefit of removing the entire identifiable tumor during a single procedure. Furthermore, experimental and preliminary clinical data indicated an increase in vascularization of metastatic disease after removing the colorectal carcinoma, and this event could enhance outgrowth of liver metastases[9]. This strategy has an important limitation, because it can be offered only in selected patients with synchronous disease[10], and this approach is associated with high rates of postoperative complications in the case of hepatic resection of advanced colorectal hepatic metastases[7].

The simultaneous resection of colorectal tumor and synchronous hepatic metastases is usually associated with good outcomes, shorter hospital stay, and reduced cost. However, simultaneous resection is accepted to be not appropriate for patients requiring major hepatic resection, elderly patients, and patients with locally advanced rectal cancer[11].

de Haas *et al*[12] studied 228 patients submitted to hepatectomy for synchronous colorectal hepatic metastases, 55 (24.1%) with a simultaneous colorectal resection and 173 (75.9%) with delayed hepatectomy. They observed disadvantages of the simultaneous strategy of complex hepatic resection associated with colorectal resection. The morbidity is not negligible, and there is some evidence that this combined strategy impacted negatively on free survival progression.

Actually, the incidence of colorectal carcinoma recurrence is higher in patients treated by the simultaneous strategy, but the three-year overall survival rates did not differ significantly concerning the surgical approach[2,3,6,7]. Furthermore, progression-free survival is significantly better when delayed hepatic surgery is performed[12]. In simultaneous strategy patients, it was observed that the morbidity rate persisted lower and the recurrence rate stayed higher, and the progression-free survival was significantly lower[12].

Chemotherapy to colorectal cancer has considerably improved with the introduction of new cytotoxic agents (oxaliplatin, irinotecan) and targeted therapies (bevacizumab, cetuximab, panitumumab)[2]. The underlying principle for the utilization of preoperative chemotherapy in these patients is to provide early treatment of metastatic disease, to decrease the recurrence rate after surgery, to assess tumor biology, to better select patients for an aggressive surgical procedure, to avoid unnecessary surgery in patients with fast-progressing disease, to test chemosensitivity of the tumor, and to tailor postoperative treatment[10]. Tumors responding to systemic chemotherapy may reflect biologically less aggressive metastases[5].

Furthermore, response to chemotherapy is now widely recognized as a major prognostic factor in patients undergoing resection of colorectal hepatic metastases[2]. The finding of tumor progression of colorectal hepatic metastases in preoperative patients under systemic chemotherapy is associated with a poor outcome, independently carrying out a curative intent surgery[13].

Taking into account the proposition that the liver metastases represent the most common cause of a patient's death, Mentha *et al*[14] described the liver-first strategy with systemic chemotherapy followed by hepatic resection of synchronous hepatic metastases and subsequent colorectal cancer resection. In this strategy, after the period of systemic chemotherapy, the colorectal liver metastases are resected before the colorectal tumor, usually after a period of downstaging chemotherapy or radio chemotherapy. This procedure was first recommended for rectal carcinoma patients with synchronous hepatic metastases, because these patients habitually required chemoradiotherapy previous to their colorectal carcinoma resection[2,11].

The liver-first strategy may represent one treatment option for patients with locally early/advanced stage rectal cancer and limited/extensive synchronous hepatic metastases. Actually, this approach should be called "chemotherapy-first" and not "liver-first" because the first approach is systemic chemotherapy that does not impair negatively on resection of the rectal carcinoma and synchronous hepatic metastases, and may downstage previously liver metastases believed unresectable[5]. Beside with these effects on synchronous colorectal hepatic metastases, the chemotherapy could downstage the primary rectal tumor. Patients with no obstructive colonic cancer with wide liver disease that necessitates downstaging may benefit from the liver-first approach[11,15].

The logical for the liver-first approach is represented by the following: major complications are uncommon in patients with stage Ⅳ colorectal cancer beneath chemotherapy; hepatectomy before the resection of colorectal carcinoma permits control of the liver metastases, making curative hepatic resection possible; subsequent resection of the primary tumor may prevent loss of primary tumor-induced inhibition of the metastases; and treatment of the metastatic disease is not postponed by radio-chemotherapy of the rectal tumor or by complications of surgical treatment of the colorectal carcinoma[2,10,11,16]. Moreover, this strategy provides a period of time that permits occult extrahepatic metastases existing to be detected[12,17].

The fact that systemic chemotherapy treats both diseases is an important advantage of the reverse strategy in patients with locally advanced colorectal carcinoma and synchronous hepatic metastases[5,18]. Mild colonic obstruction, pain, bleeding, and mucous discharge usually resolve after few a cycles of systemic chemotherapy[5]. Another advantage of the liver-first strategy is the concept that systemic metastatic disease originates from the liver's metastatic disease[6,19].

The failure to complete the liver-first approach is characterized by disease development in the liver or primary tumor, death from other comorbidities while expecting primary surgery, and morbidity and mortality succeeding liver resection[11]. When we apply the liver-first approach, there is a real jeopardy that an primarily

resectable colorectal tumor may become progressive and unresectable due to perforation or invasion into nearby structures, despite the fact that this event during induction chemotherapy is sporadic[11,15].

However, currently, the surgical pattern sequence for patients with synchronous colorectal liver metastases still remains controversial[20]. The traditional approach is staged by the limited risk of progression of colorectal liver metastases during treatment of the primary colorectal tumor. No less important is that the combined approach is only suitable for patients with not advanced or even limited metastatic liver disease. In patients with advanced metastatic disease requiring major liver resection or bilateral liver resection, chemotherapy is started first, and the reverse approach can be proposed in case of a suitable response to chemotherapy[8,20].

Brouquet *et al*[10] retrospectively analyzed the outcomes of 156 patients with synchronous colorectal hepatic metastases managed by three different surgical approaches: traditional (n = 72), combined (n = 43), and the liver-first strategy (n = 27). Patients treated with the liver-first approach had a significantly higher number and larger colorectal liver metastases than patients treated by the combined and traditional approaches. The authors reported that the postoperative mortality rates in the combined, classic, and reverse strategies were 5%, 3% and 0%, respectively, and the postoperative morbidity cumulative rates were 47%, 51% and 31%, respectively. The different surgical approaches did not exhibit different cumulative postoperative morbidity and mortality rates. There was no significant difference in 3-year and 5-year survival between the three groups, and the median disease-free survival was 11 mo in all three groups.

Andres *et al*[21] achieved a survival analysis of the liver-first reversed approach of advanced synchronous colorectal hepatic metastases based on the LiverMetSurvey with patients submitted to resection of two or more colorectal liver metastases associated with irinotecan and/or oxaliplatin-based chemotherapy before liver surgery. The authors analyzed 787 patients: 729 submitted on resection of the colorectal carcinoma, and subsequent resection of colorectal hepatic metastases all (classical approach) and 58 patients submitted on reverse strategy, which consisted of colorectal hepatic metastases directed systemic chemotherapy, resection of all hepatic metastases, and the resection of the colorectal carcinoma with neoadjuvant radiotherapy for rectal cancer. Overall survival and disease-free survival at 5 years were similar in both groups of patients.

In a systematic review about the liver-first strategy, Jegatheeswaran *et al*[6] evaluated 90 patients. They reported that disease progression during the procedure period occurred in 23 (19%) patients. de Rosa *et al*[11] reported the outcomes of 82 patients with synchronous colorectal hepatic metastases after the liver-first strategy. The authors related low global morbidity and mortality rates, with a relapse rate from 25% to 70% and an overall 5-year survival rate from 31% to 41%.

Lam *et al*[7] reported a systematic review of the liver-first strategy in patients with colorectal carcinoma and synchronous colorectal hepatic metastases. One hundred and twelve (93%) patients underwent hepatic resection of colorectal liver metastases. Eighty-nine (74%) of the initial 121 patients underwent colorectal cancer resection. They observed a post-operative morbidity of 20% and a mortality of 1% after the hepatic resection. Moreover, they related postoperative morbidity and mortality after colorectal cancer resection of 50% and 6%, respectively. In this systematic review, the overall survival was 40 mo median (range 19 to 50 mo) with a recurrence rate of 52%.

Kelly *et al*[22] described the network meta-analysis review comparing classical, combined, and liver-first approaches. These authors included 18 studies with 3605 patients in this review. Network meta-analysis and pair-wise meta-analysis of the 5-year overall survival showed no significant differences between the three surgical strategies: combined *vs* colorectal-first, liver-first *vs* colorectal-first, liver-first *vs* combined. In addition, network meta-analysis of the perioperative mortality among the three strategies was not significant. In a systematic review, Lykoudis *et al*[2] suggested that the three surgical strategies have similar results.

Despite the relatively large number of published studies on surgical strategies to synchronous colorectal hepatic metastases, there are no randomized controlled trials. The greater part of published studies is observational, usually retrospective, or non-randomized comparative studies. The identification of subgroups that could benefit from a specific strategy is a cornerstone, because outcomes are equivalent in the different approaches in the treatment of synchronous colorectal hepatic metastases[23-27].

Although the protocols used in the different studies are comparable, the liver-first strategy for patients with synchronous colorectal hepatic metastases is related with different survival results[6]. Furthermore, there is a necessity for a randomized clinical trial comparing different approaches. Factors previously considered contraindications for liver resection, such as number of metastases, synchronous metastases, and even the presence of extrahepatic disease, must not prevent the patient from having the opportunity of being treated with curative intention[16,28]. Indeed, nowadays it is accepted that even in the presence of poor prognostic factors, the possibility of long-standing survival and cure can be reached for patients with synchronous colorectal hepatic metastases[9,29,30].

The liver-first approach has been demonstrated to be safe and successful and can be an alternative in patients with locally advanced colorectal carcinoma and synchronous hepatic metastases. This approach may allow a negligible number of patients to be submitted to curative resections for the synchronous colorectal hepatic metastases and may help avoid unnecessary

surgeries in patients with incurable metastatic disease.

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REVIEW

Non-alcoholic fatty liver disease in 2015

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Abstract

There is worldwide epidemic of non-alcoholic fatty liver disease (NAFLD). NAFLD is a clinical entity related to metabolic syndrome. Majority of the patients are obese but the disease can affect non-obese individuals as well. Metabolic factors and genetics play important roles in the pathogenesis of this disorder. The spectrum of disorders included in NAFLD are benign macrovesicular hepatic steatosis, non-alcoholic steatohepatitis, hepatic fibrosis, cirrhosis of liver and hepatocellular carcinoma. Although the disease remains asymptomatic most of the time, it can slowly progress to end stage liver disease. It will be the most common indication of liver transplantation in the future. It is diagnosed by abnormal liver chemistry, imaging studies and liver biopsy. As there are risks of potential complications during liver biopsy, many patients do not opt for liver biopsy. There are some noninvasive scoring systems to find out whether patients have advanced hepatic fibrosis. At the present time, there are limited treatment options which include lifestyle modification to loose weight, vitamin E and thioglitazones. Different therapeutic agents are being investigated for optimal management of this entity. There are some studies done on incretin based therapies in patients with NAFLD. Other potential agents will be silent information regulator protein Sirtuin and antifibrotic monoclonal antibody Simtuzumab against lysyl oxidase like molecule 2. But they are still in the investigational phase.

Key words: Fatty liver; Hepatic steatosis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis

Core tip: While non-alcoholic fatty liver disease is a very common clinical problem in our day-to-day clinical practice, the management of this disease is still in its infancy. This article focuses on the epidemiology, pathogenesis, pathology, clinical presentation, investigations including noninvasive scoring systems, current treatment options and future potential agents.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a universal disorder which is now considered as the most common liver disease in the western world. NAFLD is defined as the accumulation of excessive fat in the liver in the absence of excessive drinking of alcohol and any secondary cause. Although initially benign, the disease can progress slowly from simple non-alcoholic steatosis (NAS) to non-alcoholic steatohepatitis (NASH) and subsequently to hepatic fibrosis, cirrhosis of liver and hepatoma. At the present time, there is no specific test which can predict progression of NAS to NASH. Although cirrhosis of liver secondary to hepatitis C is now the most common indication of liver transplantation in the United States, as the prevalence of NAFLD is increasing, NASH-related cirrhosis and hepatocellular carcinoma will be a major health care problem and the leading indication of liver transplantation in the future. As the epidemic of NAFLD is mainly related to insulin resistance, different therapies are now being directed to improve insulin resistance.

EPIDEMIOLOGY

Twenty percent to 30% of the general population in the western world suffer from NAFLD[1]. The prevalence is increased in type 2 diabetes mellitus (70%) and morbid obesity (90%). This correlates with the rising incidence of obesity and metabolic syndrome in the western world. In the United States, the National Health and Nutrition Examination Surveys from 2009-2010 showed obesity rates of 35.5% among men and 35.8% among women[2]. In Asia, similar prevalence of NAFLD has been found in the range of 15% to 30% in the general population and over 50% in patients with diabetes and metabolic syndrome[3]. In the general population of United States, the prevalence of NASH is about 3% but could be more than 25% in obese individuals[4].

PATHOGENESIS

Obesity is an important risk factor for the development of NAFLD. Obesity may lead to insulin resistance and metabolic syndrome which is diagnosed in the presence of 2 or more of the criteria: (1) impaired glucose tolerance (fasting blood glucose > 110 mg/dL); (2) hypertension; (3) hypertriglyceridemia (> 250 mg/dL); (4) low high density lipoprotein (HDL) level (< 40 mg/dL for men and < 50 mg/dL for women); and (5) abdominal obesity (waist > 40 inches for men and > 35 inches for women).

In fact, hepatic manifestation of metabolic syndrome is NAFLD[5]. Insulin resistance may also be responsible for the development of NAFLD even in non-obese and lean individuals. How does insulin resistance cause hepatic steatosis? Insulin suppresses lipolysis in adipose tissue. Insulin resistance in the adipose tissue leads to continued lipolysis, increased plasma free fatty acid (FFA) and FFA influx into the hepatocytes. Beta-oxidation of fatty acid is also inhibited in the liver. Other factors which play roles in hepatic lipogenesis include dietary factors, *de novo* hepatic synthesis of lipid and genetics. Dietary fat in the form of chylomicron supplies FFA to the liver. Carbohydrate metabolism leads to *de novo* synthesis of FFA from acetyl CoA. Glucose also activates carbohydrate responsive element binding protein and promotes hepatic lipogenesis. Hepatic triglyceride is generally exported into the blood as very low density lipoprotein (VLDL) with the help of apolipoprotein B (APOB). Mutation in APOB may lead to hepatic steatosis[6]. Insulin resistance can also occur in liver and skeletal muscle. Normally, insulin inhibits gluconeogenesis and promotes lipogenesis in the liver. In insulin resistant liver, gluconeogenesis continues leading to hyperglycemia and hyperinsulinemia while fatty acid synthesis is maintained in the liver. In the normal state, insulin also inhibits the production of VLDL. So in an insulin resistant state, the overproduction of VLDL in the fasting state leads to high triglyceride and low HDL in the blood. Why do obese individuals develop insulin resistance, *i.e.*, failure of insulin receptors to function? Obesity leads to hyperlipidemic and pro-inflammatory state[7]. Hepatic insulin resistance occurs when there is excess FFA influx into hepatocytes. Metabolites of FFA - long-chain acyl-CoAs and diacylglycerol - relocate cytoplasmic several protein kinase C to the membrane. Protein kinase Cs then phosphorylate intracellular portion of insulin receptors with the development of insulin resistance. It has been proposed that excessive intraperitoneal fat can cause excessive FFA reflux directly into the liver *via* the portal vein[8].

"Multiple hit" theory has been proposed in the pathogenesis of NAFLD[9]. In the first hit, there is an accumulation of triglyceride as lipid droplets within the cytoplasm of hepatocytes (steatosis) in more than 5% of hepatocytes. Insulin resistance contributes to this hepatic steatosis. This phase of benign hepatic steatosis is reversible and can be self-limited but makes the liver susceptible to the second hit which advances the liver to a necroinflammatory stage, *i.e.*, NASH. The second hit includes oxidative stress (free radical formation due to excessive fatty acid oxidation), cardiolipin (present on inner mitochondrial membrane) peroxidation leading to mitochondrial dysfunction and more reactive oxygen species formation, pro-inflammatory cytokine formation, apoptosis and gut-derived bacterial endotoxinemia.

The third hit includes palatine-like phospholipase 3 (*PNPLA3*) gene involvement, and impaired hepatocyte regeneration. A small proportion (29%) of patients with NAFLD have normal BMI. There are different genomic studies done to find out the genetic predisposition to NAFLD[10-12]. Certain single nucleotide polymorphisms (SNPs) have been found to be associated with higher frequency, severe histologic changes and more progression of NAFLD. Variant SNPs in *PZP* and *PNPLA3* genes were found to be independent risk factors for the development of NAFLD. Hence genetics play an important role along with metabolic factors in the development of NAFLD.

CLINICAL PRESENTATION

Most patients with NAFLD remain asymptomatic until they develop cirrhosis of liver when they complain of fatigue. Even before development of cirrhosis, some patients may complain of right upper quadrant discomfort or pain due to hepatomegaly and stretching of the hepatic capsule[13]. Physical examination may reveal obesity and hepatomegaly. When they develop cirrhosis of liver, they may present with cutaneous stigmata of liver disease (palmar erythema, spider nevi) or features of hepatic decompensation which include jaundice, ascites, edema, gastrointestinal bleeding and encephalopathy. Some of the clinical symptoms and signs are due to associated metabolic conditions such as diabetes mellitus, hypertension, and hyperlipidemia.

DIAGNOSIS: BIOCHEMISTRY, IMAGING AND HISTOLOGY

As most of the patients with NAFLD are free of symptoms during the pre-cirrhotic stage, they come to our attention when we find abnormal liver function tests or abnormal imaging studies done for some other reasons[13]. Abnormal liver function test with mild to moderate elevation (1.5 to 4 fold) of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and greater elevation of ALT than AST (AST/ALT: < 1) unlike alcoholic liver disease can be found in patients with NASH. Sometimes this is picked up during routine Laboratory test or during routine monitoring of statin therapy for hyperlipidemia. In fact, in the western world, NAFLD is the commonest cause of incidental abnormal liver function test (LFT)[14]. However, AST and ALT are not reliable markers of NASH as they can be normal even in advanced NAFLD. Generally, the AST:ALT ratio increases as the NAFLD advances from the necroinflammatory stage (NASH) to the fibrotic stage[15].

Imaging studies may show abnormalities suggestive of fatty liver. In clinical practice, transabdominal ultrasound is most widely used as an initial imaging modality because of its availability, low cost and no radiation exposure. Positive findings may include hyperechogenecity of the liver parenchyma, *i.e.*, bright liver relative to spleen and right kidney, hepatomegaly and blurring of vascular margins. But abdominal ultrasound cannot detect mild hepatic steatosis and cannot differentiate simple steatosis, NASH and hepatic fibrosis[16]. It is operator dependent, interfered by intra-abdominal gas and technically difficult with poor image quality in obese patients.

Non-contrast computed tomography (CT) scan may show hypodensity of the liver parenchyma as compared to spleen[17]. Contrast-enhanced CT if done on a specific protocol (time interval 2 min and liver-spleen differential of 18.5 Hounsfield units) increases the sensitivity of detection of steatosis[18].

CT involves ionizing radiation and cannot differentiate different stages of NAFLD. Transabdominal ultrasound is more sensitive than CT in detecting hepatic steatosis[19].

Magnetic resonance imaging (MRI) shows lower signal intensity of the hepatic parenchyma as compared to surrounding muscle and is more sensitive than CT scan for detection of hepatic steatosis. Hepatic triglyceride content can also be measured by MR techniques which decompose the liver signal into fat signal and water signal. Conventional MR technique (MR spectroscopy) measures the fraction of the liver signal attributable to hepatic fat. But in this technique, there can be many biological and technical confounding factors (T1 bias, T2* decay) and measurement of fat content may not be reliable[20]. New MRI technique can detect the proton density fat-fraction (PDFF) attributable to hepatic fat and thus can measure hepatic fat content directly and generally shows correlation with histologic grades of NAFLD. As the disease progresses towards fibrosis, there is less steatosis, and this can be detected by MRI-determined PDFF[21].

Histologic diagnosis of fatty liver disease by liver biopsy is the gold standard. As the histologic features of alcoholic and non-alcoholic liver disease are similar, history is very important in distinguishing these two entities. The person with NAFLD is a nondrinker or a social drinker but does not drink excessive amount of alcohol, *i.e.*, > 30 gm a day for men and > 20 gm a day for women within the last 5 years. According to Center for Disease Control and Prevention, a standard drink contains 14 gm (0.6 ounces) of pure alcohol. The standard drink could be 5 ounces of wine (12% alcohol) or 12 ounces of beer (5% alcohol) or 1.5 ounces of shot or liquor, *e.g.*, vodka, whiskey, gin, rum (40% alcohol) or 8 ounces of malt liquor (7% alcohol). As per the National Institute on Alcohol Abuse and Alcoholism, > 4 drinks on any given day or > 14 drinks per week in case of men, and > 3 drinks on any given day or > 7 drinks per week in case of women are considered heavy alcohol drinking. Thus detailed history of drinking of alcohol is very important despite the chance of inaccurate estimation. Diagnosis of NAFLD is established if there is no significant alcohol drinking history and there is fatty liver on imaging. Then the question comes whether the patient has simple steatosis, steatohepatitis, hepatic fibrosis or cirrhosis of liver. Liver biopsy is still the gold standard of finding out the histological picture of NAFLD as mentioned before.

In NAFLD, the simple steatosis is generally macrovesicular but mixed macro and microvesicular steatosis can also occur. There is fat deposition in the form of triglyceride in the cytoplasm of more than 5% of hepatocytes. In macrovesicular steatosis, the nucleus is displaced to the periphery of the hepatocyte by a single large fat globule or multiple small fat globules in the cytoplasm. In microvesicular steatosis, the nucleus remains in the center with many minute fat globules in the cytoplasm[22]. The steatosis is more prominent in the perivenular regions of the hepatocytes (zone 3). NASH is characterized by the triad of steatosis, ballooning degeneration and inflammation[23]. Ballooning

Table 1 Brunt classification of steatohepatitis[27]

Grades of NASH	
Grade 1 (mild)	Steatosis up to 66%. Occasional ballooned hepatocytes predominantly in zone 3. Scattered intra-acinar neutrophils
Grade 2 (moderate)	Steatosis of any degree. Ballooned hepatocytes predominantly in zone 3. Intra-acinar neutrophils. Zone 3 perisinusoidal fibrosis. Mild to moderate portal and intra-acinar chronic inflammation
Grade 3 (severe)	Panacinar steatosis. Widespread ballooned hepatocytes predominantly in zone 3. Intra-acinar inflammation. Scattered neutrophils associated with ballooned hepatocytes. Mild to moderate portal inflammation
Stages of NASH	
Stage 1	Extensive zone 3 perisinusoidal fibrosis
Stage 2	Zone 3 perisinusoidal and portal or periportal fibrosis
Stage 3	Bridging fibrosis
Stage 4	Cirrhosis

NASH: Non-alcoholic steatohepatitis.

Table 2 Non-alcoholic steatosis or non-alcoholic fatty liver disease activity score is determined by evaluating the steatotic and inflammatory activity

NAS	Steatosis	Ballooning	Inflammation, lobular
0	< 5% (0)	None (0)	None (0)
3	5%-33% (1)	Rare or few (1)	1–2 foci per 20 × field (1)
6	34%-66% (2)	Many (2)	2–4 foci/20 × field (2)
8	> 66% (3)	Many (2)	> 4 foci/20 × field (3)

NAS: Non-alcoholic steatosis.

degeneration also considered as the hallmark of steatohepatits is recognized by a swollen hepatocyte with foamy, pale cytoplasm and enlarged hyperchromatic nucleus. Loss of normal hepatocyte keratins 8/18 immunostaining can be helpful in the detection of the ballooned hepatocytes[24]. Mild inflammation mainly involving the acini and sometimes the portal tract is the central feature in NASH. Mixed inflammatory cells consisting of lymphocytes, plasma cells, monocytes, eosinophils and neutrophils are found. Ballooned hepatocytes surrounded by neutrophils, a lesion called "satellitosis" can be rarely seen in NASH. Sometimes, intracytoplasmic inclusions (ubiquitin-rich) called Mallory's hyaline are found in the hepatocytes. As the disease progresses, portal inflammation becomes more severe. Hepatic fibrosis generally begins in zone 3. There is pericellualr and perisinusoidal fibrosis giving characteristic "chicken wire" appearance. Portal and periportal fibrosis occurs as well. Then bridging fibrosis with central to portal, and central to central fibrous septa formation is seen, ultimately leading to macronodular or mixed cirrhosis of liver. At this stage, the characteristic triad of NASH and perisinusoidal fibrosis becomes less prominent or disappear. As a result, many times NASH-related cirrhosis are labeled as cryptogenic cirrhosis. This may lead to hepatic failure and hepatoma. One study showed that the chance of developing hepatoma in patients with cirrhosis secondary to NAFLD was 7% over 10 years time period[25]. Non-cirrhotic NAFLD patients may also develop hepatoma possibly because of associated metabolic syndrome[26].

As mentioned before that NAFLD is a spectrum of disorders:

Simple steatosis → NASH → cirrhosis

NAFLD classification: type 1: Simple steatosis; type 2: steatosis + inflammation (lobular and portal) → NASH; type 3: steatosis + ballooned hepatocytes → NASH; type 4: steatosis + fibrosis → NASH.

Grades of hepatic steatosis: Hepatocytes containing fat vacuoles are subjectively visualized and graded. grade 0 (normal): < 5% of hepatocytes are affected; grade 1 (mild): 5% to 33% of hepatocytes are affected; grade 2 (moderate): 34% to 66% of hepatocytes are affected; grade 3 (severe): > 66% of hepatocytes are affected (Table 1).

NAS or NAFLD activity score is determined by evaluating the steatotic and inflammatory activity as Table 2.

Although liver biopsy is widely available and very helpful in staging and grading NAFLD, it is an invasive procedure with inherent risks of complications like pain at the biopsy site, intraperitoneal bleeding, subcapsular hematoma, infection and accidental injury to other organs. After liver biopsy, patients may need to stay at the hospital for several hours for recovery. Rarely (1%-3% of cases), patients may need to get admitted to the hospital and the mortality is 1 in 10000[28]. Many patients are also reluctant to have liver biopsy done. As advanced hepatic fibrosis can eventually lead to cirrhosis of liver and hepatoma, assessment of patients with NAFLD and hepatic fibrosis is important (Table 3).

The Fibrosis 4 index was found to be superior when comparison was made among the non-invasive markers of fibrosis in patients with NAFLD.

Hepatic fibrosis can also be evaluated by hepatic elastography which measures liver stiffness. Hepatic elastography can be done by ultrasound or MRI[34]. In ultrasound elastography also known as Fibroscan or Transient Elastography, a transducer on an ultrasound probe transmits ultrasound wave (50-MHz) into the liver which then produces an elastic shear wave (meter/sec). The shear wave passes faster through the fibrous tissue. The shear wave is then converted into liver stiffness (kilopascols)[35]. Fibroscan is very sensitive (70%) and specific (84%) in detecting the stages of hepatic fibrosis[36]. There are some technical issues

Table 3 Several noninvasive scoring systems based on indirect serologic markers of fibrosis are available to predict the presence or absence of advanced hepatic fibrosis

BARD (BMI > 28, AST/ALT ⩾ 0.8 and diabetes mellitus) score[29]: Score ranges from 0 to 4. BMI > 28 (yes = 1, no = 0) + AST/ALT (> 0.8 = 2, ⩽ 0.8 = 0) + diabetes mellitus (yes = 1, no = 0)	Score 0 to 1 means low probability of advanced hepatic fibrosis (negative predictive value 96%) and score 2 to 4 means high probability of hepatic fibrosis (positive predictive value 43%)
NAFLD fibrosis score: depends on age, BMI, diabetic status, AST, ALT, Platelet Count and albumin[30]: -1.675 + 0.037 × age (yr) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × Platelet (10^9/L) - 0.66 × albumin (g/dL)	If the score is < -1.455, there is low probability of advanced hepatic fibrosis (negative predictive value ⩾ 87%) and if the score is > 0.676, there is high probability of advanced hepatic fibrosis (positive predictive value ⩾ 78%). If the score is intermediate (between -1.455 and 0.676), there is indeterminate probability and these patients need to have liver biopsy for further assessment
Fibrosis 4 index: Uses age, AST, ALT and platelet count[31]: Age (yr) × AST (U/L)/platelet (10^9/L) × [ALT (U/L)]$^{1/2}$	If the score is < 1.30, there is low probability of advanced hepatic fibrosis (negative predictive value 90%), if the score is > 2.67, there is high probability of advanced hepatic fibrosis (positive predictive value 80%). If the score is intermediate (1.30 to 2.67), the possibility of having advanced hepatic fibrosis is indeterminate and liver biopsy is warranted
APRI[32]: AST level (IU/L)/AST upper limit of normal (IU/L)/[platelet count (10^9/L)] × 100 =	If the score is ⩽ 0.5, there is low probability of hepatic fibrosis negative predictive value 83% and if the score is > 1.5, there is high probability (positive predictive value 68.4%) of hepatic fibrosis[33]. The intermediate score is indeterminate and liver biopsy should be done in those patients

BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Non-alcoholic fatty liver disease; IFG: Impaired fasting blood glucose; APRI: AST platelet ratio index.

which limit performance of doing Fibroscan, including morbid obesity, ascites, narrow intercostal spaces and excessive chest wall fat.

MR elastography (MRE) has a vibration device which produces shear waves in the liver. The shear waves are detected by the modified MRI machine, generating a color image (elastogram) that represents wave velocity and hence stiffness of the liver. MRE is superior in differentiating different stages of fibrosis (sensitivity 85.4%, specificity 88.4%)[37]. The limitations will be cost and claustrophobia.

MANAGEMENT

The goal of management will be to diagnose the disease early, prevent further progression of the disease from one stage to the next stage, regression of the disease as much as possible and improvement of the underlying metabolic syndrome. When the patient becomes cirrhotic, standard treatment of cirrhosis should be offered including liver transplantation in the decompensated state. NAFLD can recur in the transplanted liver.

Lifestyle modification

As most of the patients with NAFLD are overweight or obese and have associated metabolic syndrome, gradual weight loss is advocated as the first line of intervention[38]. Diet and exercise (30 min of aerobic exercise 4 times a week, *i.e.*, moderate physical activity) are the preferred methods of weight loss. There are many studies showing the benefit of weight loss in NAFLD[39]. Five percent to 10% of body weight loss can reduce a significant amount of liver fat and improve steatohepatitis. But as it is difficult to maintain body weight, many patients regain lost body weight with the recurrence of NAFLD. Dietary modification is also very important. High sugar consumption in the Western diet is the major cause of obesity. Diet rich in fructose particularly high fructose corn syrup (Granola bars, condiments, sweetened beverages, prepared desserts, baked goods, snacks, breakfast cereal, cookies) may impair insulin sensitivity leading to development of NAFLD[40]. Thus sugar consumption should be less than 10% of one's total caloric intake and food rich in high fructose corn syrup should be avoided. Western diet is also rich in saturated fat and omega-6 fatty acid but deficient in omega-3 fatty acid[41]. Omega-3 fatty acids normally coordinate with upregulation of fatty acid oxidation and downregulation of fatty acid synthesis. Dietary omega-3 fatty acid deficiency associated with increase in omega-6 fatty acid in the body has been found to cause NAFLD in rats and mice. Cooking oils high in omega-6 fatty acid (soybean, sunflower, corn) should be changed to cooking oils high in omega-3 fatty acids (Canola, Olive, Chia, Perilla). Patients should be encouraged to eat more fish as they contain omega-3 fatty acid. Fish oil supplementation helps in improving the lipid profile and reducing the inflammatory markers of metabolic syndrome[42] although further studies are needed to find out its beneficial effects on metabolic syndrome. One study showed diet and exercise were superior to insulin sensitizers metformin and rosiglitazone in ALT normalization in NAFLD[43].

Pharmacotherapy

As NAFLD is associated with metabolic syndrome, the associated comorbidities like obesity, diabetes mellitus, hypertension and hyperlipidemia should be managed well concurrently as part of the treatment of NAFLD. There is a practice guideline developed by American Association for the study of Liver Diseases and approved by American College of Gastroenterology and the American Gastroenterological Association on the management of NAFLD. The guideline was published in *Hepatology* in 2012[44].

The broad categories of pharmacotherapy for the treatment of NAFLD include: (1) Antioxidants; (2)

Insulin-sensitizing agents; (3) Hepatoprotective and miscellaneous agents; and (4) Bariatric surgery.

Antioxidants

As oxidative stress is considered to be the main mechanism of progression of steatosis to steatohepatits, the antioxidant Vitamin E has been studied in different trials. Vitamin E 800 units per day was studied in the PIVENS trial[45]. It showed improvement in steatosis and steatohepatitis and decrease in serum transaminases in nondiabetic patients but there was no improvement of fibrosis histologically. Currently it is recommended as the first line agent in nondiabetic individuals with biopsy proven NASH.

INSULIN SENSITIZING AGENTS

Metformin

Metoformin is a common and first line antidiabetic agent as it increases insulin sensitivity by upregulating AMP-activated protein kinase which results in the reduction of hepatic glucose production[46]. Although there was initial enthusiasm about Metformin on its therapeutic effect on NAFLD, subsequent studies did not find much benefit. A pilot study showed little effect of Metformin on serum transaminases and liver histology in NAFLD[47]. Currently metformin is not recommended as a specific treatment of NAFLD.

THIOGLITAZONES

Thioglitazones (Pioglitazone and Rosiglitazone) are agonists of peroxisome proliferator-activated receptor gamma that controls transcription of insulin receptor genes involved in the transport, utilization and production of glucose and lipid[48].

These nuclear receptors are found in liver, muscle and fat cells. Thioglitazones act as insulin sensitizers in NAFLD by helping to redistribute fat from the liver and muscles to the adipose tissue. In the PIVENS trial[49], pioglitazone improved serum transaminases, steatosis and steatohepatitis in nondiabetic patients with NASH but histological improvement was not statistically significant in comparison to placebo. Thioglitazones can cause weight gain and carry increase risk of congestive cardiac failure. At the present time, thioglitazones can be recommended to treat NASH, but long term safety and efficacy are not known.

MISCELLANEOUS AGENTS

Ursodeoxycholic acid

A naturally occurring secondary bile acid found in small quantities in the human small intestine, is produced by intestinal bacteria as a metabolic by-product and it is found in large quantities in the bile of certain types of bear. It has cytoprotective effects along with the ability to alter lipid properties. The acid can reduce transaminases in NAFLD[50] but long-term study failed to improve any liver histology[51,52]. As a result, ursodexycholic acid is not a treatment option for NAFLD.

Pentoxifylline

Pentoxifylline is a xanthine derivative and is being used in peripheral vascular disease because of its beneficial effects like relaxation of smooth muscle, flexibility of red blood cells and deaggregation of platelets. Because of anti-tumor necrosis factor activity, it has been used in alcoholic hepatitis, and studied in NAFLD. A randomized placebo controlled trial by Zein *et al*[53] showed that pentoxifylline 400 mg 3 times a day over 1 year improved steatosis and lobular inflammation with no significant effect on ballooning degeneration[53]. However, in a similar study done by Van Wagner *et al*[54], pentoxifylline improved transaminases, hepatic steatosis and ballooning degeneration when compared to baseline but when compared to placebo, the improvement was not clinically significant. Pentoxifylline did not improve any metabolic marker of insulin resistance. These findings warrant further studies to determine the role of pentoxifylline in NAFLD.

Statins

NAFLD and hyperlipidemia frequently coexist as part of the metabolic syndrome. Statins are used as one of the main line therapies for hyperlipidemia. Statins may cause mild elevation of transaminases but they have been found to be safe in patients with chronic liver diseases including NAFLD[55]. One randomized study showed that Atorvastatin improved both biochemical and ultrasound evidence of NAFLD[56]. But at the present time, there is no randomized controlled study evaluating the effect of statin on the histology of NAFLD. Statins are not currently recommended specifically for the treatment of NAFLD.

Omega-3 fatty acids

In the western diet, omega-6 fatty acid consumption is high and omega-3 fatty acid consumption is low - a phenomenon that may lead to an increased amount of pro-inflammatory arachidonic acid derivatives (eicosanoids) production and impaired hepatic lipid metabolism, predisposing to NAFLD. A meta-analysis showed treatment with omega-3 polyunsaturated fatty acid improved hepatic steatosis but not transaminases but the correct dose is currently not known[57]. Further randomized controlled trials are needed. At the present omega-3 fatty acid supplementation is not recommended for the treatment of NAFLD.

Orlistat

Orlistat is a reversible enteric and pancreatic lipase inhibitor. It promotes fat malabsorption, and decreases free fatty-acid influx into the liver leading to weight loss and improvement of insulin sensitivity. In a randomized controlled trial, Orlistat reduced serum transaminases and hepatic steatosis as determined by abdominal ultrasound[58]. Another study demonstrated that significant

weight loss of > 9% improved serum transaminases and liver histology irrespective of intake of Orlistat[59]. Currently, Orlistat is approved for weight loss in obese patients but not recommended solely for the treatment of NAFLD.

Incretin-based therapies

Glucagon-like peptide 1 (GLP-1) secreted by the L cells of the intestinal mucosa after nutrient ingestion is an incretin hormone. It increases insulin secretion by stimulating pancreatic β cells, decreases glucagon secretion and delays gastric emptying. Thus it lowers blood glucose in diabetes mellitus and has other beneficial effects including central appetite suppression, weight reduction and improvement of insulin sensitivity[60]. Because of rapid degradation by dipeptidyl-peptidase Ⅳ (DPPⅣ), GLP-1 has a short half life. GLP-1 receptor agonists (exenatide, liraglutide) are long acting as they are DPP Ⅳ resistant. They are primarily developed for type 2 diabetes mellitus for maintenance of blood glucose. There are case reports in which diabetic patients with NAFLD when treated with exenatide showed significant decrease in liver fat. In obese mouse, exendin-4 improved insulin sensitivity and reversed hepatic steatosis[61]. Hepatic DPP Ⅳ expression and serum DPPⅣ activity are significantly higher in NAFLD patients and they correlate with hepatic steatosis[62]. DPPⅣ inhibitor sitagliptin treated diabetic NAFLD patients displayed a decrease in transaminases and hepatic steatosis[63,64]. Thus considering the experimental and clinical data, incretin-based therapies (GLP-1 analogues and DPPIV inhibitors) can be considered as potential novel agents in the treatment of NAFLD. Further randomized controlled trials are needed before starting incretin-based therapies as therapeutic agents for NAFLD.

BARIATRIC SURGERY

Most of the patients who undergo bariatric surgery have NAFLD. Common bariatric surgeries practiced in the United States are Roux-en-Y gastric bypass, laparoscopic adjustable gastric banding, sleeve gastrectomy, and biliopancreatic diversion with duodenal switch[65]. Steady and profound weight loss increases insulin sensitivity, promotes visceral fat loss and can potentially improve liver histology in NAFLD. Although beneficial effects including improved liver histology were seen in few studies, a randomized controlled trial that has evaluated bariatric surgery as the treatment of NAFLD has not been pursued. There is concern of hepatic failure in cirrhotic patients due to rapid weight loss[66]. Bariatric surgery in cirrhosis of liver due to NAFLD could be risky. Although bariatric surgery is frequently done in morbidly obese individuals with non-cirrhotic NAFLD to reduce obesity, it is not recommended as a primary treatment for NAFLD.

FUTURE THERAPY

Research is ongoing to find out prevention and better therapeutic options of NAFLD. Sirtuins (SIRTs) are silent information regulator proteins which act as nicotinamide adenine dinucleotide dependent deacylases and thus can modulate activation and deactivation of certain proteins[67]. In mammals, there are 7 different types of SIRT 1-7. SIRT1 has been found to increase insulin sensitivity and secretion, decrease oxidative stress and inflammatory activity, and help in glucose and lipid metabolism. In the rat model, significantly decreased SIRT expression in the liver was found in NAFLD and moderate SIRT1 overexpression in the liver was protective from developing NAFLD[68]. In another murine model, resveratrol, a natural SIRT1 activator, showed improvement of insulin resistance and liver histology in NAFLD[69]. Thus pharmacological activation of SIRT1 can be a potential target in the treatment of NAFLD but human studies (randomized controlled trials) are needed.

Hepatic fibrosis at a more advanced stage leads to cirrhosis of the liver. Lysyl Oxidase Like Molecule 2 (LOXL2) is an enzyme that causes cross linkage of type 1 collagen and promotes fibrosis[70]. Its serum level correlates with the stage of hepatic fibrosis[71]. Simtuzumab is a humanized antifibrotic monoclonal antibody (IgG4) against LOXL2. It was well tolerated in patients with liver disease of diverse etiology in a small study[72]. In multicenter clinical trials, Simtuzumab is currently being evaluated for its safety and efficacy in patients with compensated cirrhosis due to NASH, and also in patients with advanced hepatic fibrosis but not cirrhosis secondary to NASH[73].

PROGNOSIS

Most of the patients with NAFLD will die from cardiovascular events. Simple steatosis has a benign course and can be reversible. NASH is a progressive disease leading to hepatic fibrosis and ultimately cirrhosis of the liver in 20% of the time. The chance of developing hepatoma is also high in NAFLD, particularly in cirrhotic liver. Besides the liver disease, the associated components of metabolic syndrome give rise to morbidity and mortality. Cardiovascular disease, cancer and cirrhosis are the top three causes of death[74]. Recently a long-term (> 12 years) international study found that although lean patients (body mass index < 25 kg/m^2) with NAFLD had less insulin resistance and less advanced hepatic fibrosis, they had twice (28% *vs* 14%) the mortality than their overweight and obese counterparts[75].

CONCLUSION

NAFLD is the most common cause of incidental abnormal LFT, and the most prevalent chronic liver disease in the world. Because of the epidemic of NAFLD, it is predicted to be the commonest indication of liver transplantation in the near future. Good preventive measures, better understanding of the underlying mechanisms of the disease, reliable non-invasive diagnostic tests and

effective therapies are essential for optimal management of the disease. At the present time, we have practical guidelines but only few options which include life-style modifications to achieve targeted weight loss, vitamin E and pioglitazone in non-diabetic patients with biopsy-proven NASH. Although metabolic syndrome plays a major role in most of the patients with NAFLD, the pathogenic mechanism is heterogenetic as evidenced in the recent finding of higher mortality in lean NAFLD patients who are more likely to be men, non-white, especially Asian and Hispanic, with few metabolic conditions like diabetes, hypertension, hyperlipidemia, less elevated transaminases and less fibrosis. In future, treatment should be more individualized depending on the underlying pathogenic mechanism.

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REVIEW

Multidisciplinary perspective of hepatocellular carcinoma: A Pacific Northwest experience

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Abstract

Hepatocellular carcinoma (HCC) is the most rapidly increasing type of cancer in the United States. HCC is a highly malignant cancer, accounting for at least 14000 deaths in the United States annually, and it ranks third as a cause of cancer mortality in men. One major difficulty is that most patients with HCC are diagnosed when the disease is already at an advanced stage, and the cancer cannot be surgically removed. Furthermore, because almost all patients have cirrhosis, neither chemotherapy nor major resections are well tolerated. Clearly there is need of a multidisciplinary approach for the management of HCC. For example, there is a need for better understanding of the fundamental etiologic mechanisms that are involved in hepatocarcinogenesis, which could lead to the development of successful preventive and therapeutic modalities. It is also essential to define the cellular and molecular bases for malignant transformation of hepatocytes. Such knowledge would: (1) greatly facilitate the identification of patients at risk; (2) prompt efforts to decrease risk factors; and (3) improve surveillance and early diagnosis through diagnostic imaging modalities. Possible benefits extend also to the clinical management of this disease. Because there are many factors involved in pathogenesis of HCC,

this paper reviews a multidisciplinary perspective of recent advances in basic and clinical understanding of HCC that include: molecular hepatocarcinogenesis, non-invasive diagnostics modalities, diagnostic pathology, surgical modality, transplantation, local therapy and oncological/target therapeutics.

Key words: Genetic alterations; Epigenetic alterations; Diagnostic pathology; Diagnostic imaging; Surgical modality; Liver transplantation; Locoregional therapy; Sorafenib; Hepatocellular carcinoma; Liver resection

Core tip: Hepatocellular carcinoma (HCC) is one of the few tumors in which the incidence is on the rise worldwide, especially in the United States. The overall increase in the incidence warrants efforts to prevent and to more efficiently treat this disease. This necessitates the need for a multidisciplinary approach for the management of HCC, because there are many etiological factors involved in the pathogenesis and malignant transformation of the disease. For example, there is a need to improve surveillance and early diagnosis through diagnostic imaging modalities to facilitate identification of potential molecular targets for novel therapeutic strategies. In turn, this will facilitate the identification of patients at risk. This review summarizes current knowledge on the clinical management of the disease as well as etiologic mechanisms of malignant transformation for better diagnosis, prognosis, and treatment of HCC.

Yeh MM, Yeung RS, Apisarnthanarax S, Bhattacharya R, Cuevas C, Harris WP, Hon TLK, Padia SA, Park JO, Riggle KM, Daoud SS. Multidisciplinary perspective of hepatocellular carcinoma: A Pacific Northwest experience. *World J Hepatol* 2015; 7(11): 1460-1483 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i11/1460.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i11.1460

INTRODUCTION

Hepatocellular carcinoma (HCC) constitutes the majority of primary liver cancers. It is the fifth most common malignancy in the world and is the third leading cause of cancer-related death worldwide[1,2]. More than half a million cases are newly diagnosed each year, with an almost equal annual mortality given its high fatality rates. The incidence of HCC continues to rise and is predicted to continue to be the third cause of cancer-related death by 2030[3]. Viral hepatitis and cirrhosis are known to be the most common risk factors for HCC, but the exact mechanisms of hepatocarcinogenesis remain unclear, particularly in patients without these risk factors. Fatty liver disease due to diabetes and obesity has recently been recognized as independent risk factor of HCC, but may also act synergistically with other risk factors such as viral hepatitis to contribute to the processes of hepatocarcinogenesis[4-6].

The treatment options for HCC include those of curative potential for early stage of the disease such as surgical resection, ablation, and liver transplantation. The only therapies that have been shown to prolong life for intermediate or advanced stage disease include liver-directed therapy with transarterial chemoembolization and systemic chemotherapy with sorafenib. As HCC is a complex type of cancer, optimal management requires a multidisciplinary-team approach including oncological surgeons, hepatologists, oncologists, radiologists, intervention radiologists, transplant surgeons, and pathologists, who routinely meet and discuss diagnosis and treatment options towards individualized management with the goal fulfilling precision medicine. This review aims to discuss the current understanding of the mechanisms and signaling pathways involved in hepatocarcinogenesis, pathological and radiological diagnosis, and management of HCC with a multidisciplinary approach.

GENETIC ALTERATIONS IN HCC

The genetic heterogeneity of HCC has complicated the search for driver mutations that initiate or promote HCC. Technological advancements in genomic research over the past decade, such as whole exome sequencing (WES), whole genome sequencing (WGS), and whole transcriptome analysis, have allowed more extensive genomic analyses of HCC. This section will focus on common genetic and epigenetic mutations in HCC, molecular classification of HCC, and the signaling pathways that may serve as therapeutic targets.

Multiple groups have performed whole exome as well as WGS of HCC in order to determine the most common genetic mutations involved in this disease[7-9]. Cleary *et al*[7] performed WES of 87 tumors and found that the most frequent mutations were TP53 (18%), CTNNB1 (10%) and MLL4 (7%) among others. Their work demonstrates the heterogeneity of HCC as they had a relatively even distribution of hepatitis C virus (HCV), HBV, and cirrhosis not otherwise specified in their cohort and no single mutation was present in > 20% of samples. Fujimoto *et al*[8] performed WGS on 27 tumors, all but two-harbored HCV or HBV. They also found TP53 to be the most frequent mutation (14/27 samples). Their work also found mutations in CTNNB1, MLL, as well as ARID1a/2. Kan *et al*[9] performed WGS on 88 tumors, 81 of which were positive for HBV. Their group also found TP53 as the most frequent mutation (35%) followed by CTNNB1 (16%) and JAK1 (9%). Pathway analysis was used to describe five major cellular pathways that are altered by the somatic mutations found by WGS: (1) P53/cell cycle; (2) Wnt/β-catenin (CTNNB1); (3) Chromatin remodeling (ARID); (4) PI3K/AKT/mammalian target of rapamycin (mTOR); and (5) Oxidative/ER stress. Overall, specific mutations were present in < 20% of all samples, further illustrating the genetic heterogeneity found in HCC (for further

Table 1 Genes frequently mutated in hepatocellular carcinoma

Gene	Pathways/gene functions involved	Ref.
p53	Genome integrity and cell cycle	Clearly *et al*[7]
CTNNB1	Wnt/β-catenin signaling	Kan *et al*[9]
ARID1A	Chromatin remodeling	Fujimoto *et al*[8]
mTOR	PI3K/AKT/mTOR	Riechle *et al*[20]
NFE2L2	Oxidative/ER stress	Guichard *et al*[16]
TERT promoter	Telomere stability	Nault *et al*[183]

PI3K/AKT/mTOR: Phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin.

information about HCC genetic dysregulation in Table 1).

The great diversity of genetic alterations in HCC reflects the multiple etiologic factors that contribute to its pathogenesis. It is well known that HBV, HCV, alcoholic cirrhosis, aflatoxin-B, non-alcoholic steatohepatitis (NASH) and hemochromatosis all portend a higher risk of developing HCC. Multiple groups have used WES and WGS as well as genome wide association studies to define genetic "signatures" for different etiologies of HCC[10-12]. For example, those associated with alcoholic cirrhosis tend to have increased mutations in the chromatin-remodeling pathway, HCV-associated HCC were shown to have increased rates of CTNNB1 mutations (Wnt/β-catenin pathway) and ARID2 mutations (chromatin remodeling complex). In contrast, HBV-related tumors are commonly caused by integration of the viral HBx DNA into the host genome, which creates genetic instability and mutagenesis in cancer related genes such as TP53. Multiple groups have found common integration sites in the promoter sites or exons of TERT (MLL4), CCNE1, and ROCK1 genes that are significantly increased in HBV associated HCC. They also have a higher rate of differentially regulated TP53[13,14]. Aflatoxin B1 exposure results in a predictable mutation in codon 249 of TP53 which drives carcinogenesis[15]. Guichard *et al*[16] described a novel mutation in IRF2 that is present in HBV-related HCC which leads to TP53 inactivation. A recent study by Lau *et al*[17] found a common viral-human chimeric transcript resulting from HBV integration into a LINE1-element on chromosome 8p11.21, which functions as a long non-coding RNA to drive oncogenesis through its influence on Wnt/β-catenin pathway. Zain *et al*[18] have used genome wide analysis of copy number variation to identify rare or novel copy number variant that are associated with progression of NASH to cirrhosis and eventually HCC. There is, however, a paucity of data regarding the genetics of NASH and HCC, and given the worldwide rise in prevalence of NASH this presents a significant gap in our knowledge of the HCC cancer genetics.

Certain subtypes of HCC, however, have been associated with single driver mutations. Recently, WGS of fibrolamellar carcinoma has revealed a chimeric transcript of DNAJB1-PRKACA that is present in all tumor samples studied[19]. This rare variant of HCC occurring in young adults without cirrhosis also shows involvement of the mTORC1 and FGFR1 pathways[20].

There are also genetic signatures for clinical characteristics as well as risk assessment for certain HCCs. For example, Cleary *et al*[7] found that increased microvascular invasion was associated with MLL mutations, and those tumors with TP53 mutations were at higher risk for early recurrence. Multiple groups have also discovered various SNPs and their association with HCC risk[21]. Budhu *et al*[22] created 17-gene profile that was able to predict tumor metastasis and recurrence in an independent cohort. Huang *et al*[23] used WES of HBV related HCC with associated portal vein tumor thrombus (PVTT). They discovered novel mutations present only in the PVTT that suggest they may be involved in tumor progression[23].

The cumulative genetic and epigenetic alterations lead to changes in gene expression in HCC. Many groups have sub-classified HCC based on their transcriptome profile. Hoshida et al classified HCC into three groups, S1-S3. S1 tumors tend to have mutations in the Wnt/β-catenin pathway, have increased risk of early recurrence, and have increased vascular invasion as well as satellite lesions. S2 tumors are those with activating mutations in the MYC and PI3K/AKT pathways. They tend to be larger tumors that overexpress AFP. S3 tumors are well differentiated, have fewer inactivating mutations of p53, and tended to be smaller tumors[24]. Boyault's group used whole transcriptome analysis of 103 HCC samples to classify six subgroups of HCC. G1-G3 groups were associated with increased genomic instability. G1-G 2 groups were both found to have AKT activation and were associated with HBV. For G1 groups, tumors had low copy numbers of HBV, while G2 tumors had higher copy numbers of HBV as well as TP53 and PIK3CA mutations. Tumors for G3 group were classified by having TP53 and cell cycle pathway mutations, whereas tumors for G4 group were heterogeneous, mostly with TLF-1 mutations. For G5-G6 groups, tumors were found to carry Wnt/β-catenin mutations such as CTNNB1. They also exhibited decreased expression of cell adhesion proteins and tended to have increased satellite lesions[25].

Epigenetic changes in HCC carcinogenesis and prognosis have also been investigated. There are a myriad of differentially regulated miRNA, alterations in DNA methylation, and dysregulations of histone complexes that occur in HCC. The complete list of miRNA is beyond the scope of this review. Seemingly, the most clinically relevant are the Let-7 miRNA, which are down regulated in HBV related HCC. Also, miRNA-196 seems to have a protective role in HCV related HCC. miRNA-26a and 195 are both down regulated inn HCC, which leads to decreased E2F expression and cell survival/proliferation[26-28].

DNA hypo-and hypermethylation can lead to differential regulation of tumor suppressors and oncogenes. In HCC, multiple groups have identified hypomethylation (activation) of oncogenes such as LINE-2, ALU, STAT2 as well as hypermethylation (suppression) of RB1, P16, APC, SOCS1, SOCS3, and RASSF1a. DNA methylation

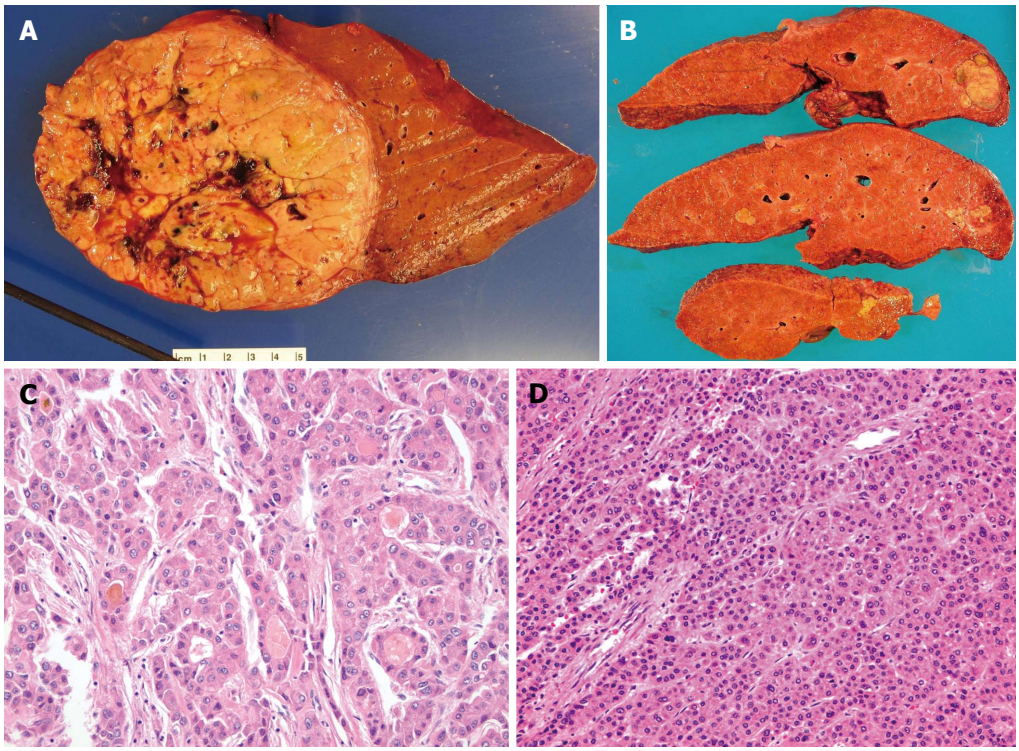

Figure 1 Pathology of classical hepatocellular carcinoma. A: Gross photo of a well circumscribed, soft, yellowish to tan, and lobulated hepatocellular carcinoma (HCC) in a background of non-cirrhotic liver; B: Gross photo of a yellow and greenish, soft and lobulated HCC in a background of cirrhotic liver; C: Microphotos of HCC showing the pseudoacinar and pseudoglandular patterns, some containg the yellowish bile within the pseudoglandular structure with increased nuclear sizes; D: Microphotos of HCC showing thickened trabeculi, with increased unpaired arteries. Notice there are no normal structures present, *i.e.*, portal tracts.

abnormalities occur early in the course of HCC, and seem to accumulate as the disease progresses[29]. Nagashio's group has identified specific methylation signatures that differentiate malignant HCC from benign lesions with > 95% sensitivity and specificity. They have also shown that certain methylation sites such as RIZ1a and LINE-1 may have prognostic value[30].

The advent of WGS and WES has uncovered a myriad of novel mutations found in HCC. More studies are needed to define their role in hepatocarcinogenesis. This could lead to further targets for therapy, risk stratification, as well as development of biomarkers for early detection of HCC. As the technology improves we may be able to personalize targeted therapy for specific mutational profile found in each tumor.

PATHOLOGY OF HCC

Pathology has played an important role in the diagnosis, staging and follow-up for the management of HCC. HCC is a morphologically heterogeneous tumor. Grossly HCC appears as circumscribed, yellow to greenish and soft tumors, often encapsulated with areas of hemorrhage and necrosis. Infiltrative borders can be seen but are not common. The background liver may or may not be cirrhotic (Figure 1A and B). Histologically HCC can show range of differentiation from well, moderate to poor, with a spectrum of architectural patterns including trabecular (greater than two cells in thickness yet still resembling hepatocytic cords), pseudoglandular or pseudoacinar to solid (Figure 1C). Normal structures, *i.e.,* portal tracts are not present within the neoplastic tissue, where the blood is solely supplied by the artery; hence "unpaired arteries" are increased within HCC (Figure 1D), a phenomenon reflecting the neoangiogenic property during hepatocarcinogenesis and the hypervascularity observed by imaging. Sinusoidal capillarization is also a unique characteristic of HCC in which fenestrated hepatic sinusoids transform into continuous capillaries. In HCC, the neoplastic cells can appear similar to hepatocytes to markedly pleomorphic or small cell and undifferentiated. Features of hepatocytic differentiation may still retain in the neoplastic hepatocytes, such as bile, glycogen, steatosis, and Mallory-Denk bodies. Malignant features including enlarged and vesicular nuclei with prominent nucleoli are often seen. Mitotic figures are frequent and can appear bizarre in the poorly differentiated tumor. Although not always present, stromal invasion is a malignant feature of HCC that can be used to distinguish HCC from dysplastic nodule, where loss of ductular reaction by keratin 7 (CK7) or CK19 is observed by immunohistochemistry in HCC[31,32]. Reticulin stain has been traditionally useful to diagnose HCC, in which the thickened trabecula are highlighted by the loss of reticulin stain. In HCC, the sinusoidal endothelial cells stain positive for CD34[33,34], whereas they are negative in the non-neoplastic liver tissues. While glypican-3 is also a relatively sensitive and specific marker for HCC, the

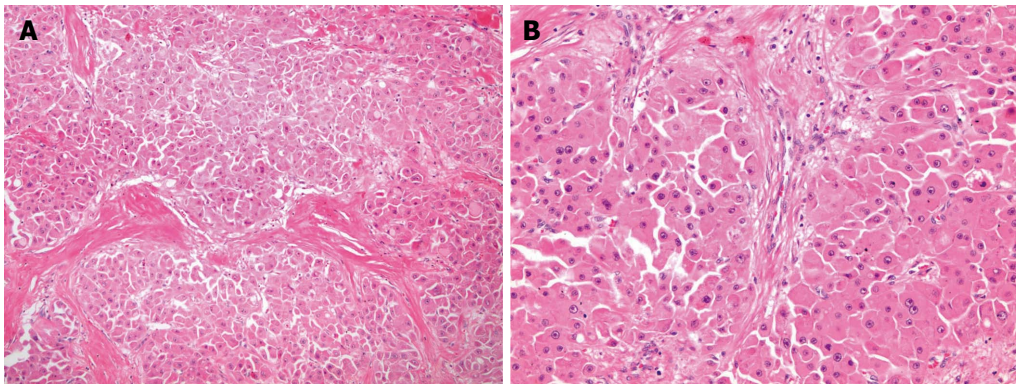

Figure 2 Pathology of fibrolamellar carcinoma. A: Microphotos of fibrolamellar carcinoma showing the thick lamellar bands of fibrosis under low power magnification; B: In higher power magnification, the tumor cells are large and polygonal with abundant eosinophilic and granular cytoplasm, large vesiculated nuclei, and prominent nucleoli.

staining may be focal, and its sensitivity may decrease in well differentiated HCC, thus cautious interpretation is warranted[35,36]. Hepatocytic differentiation of HCC can be demonstrated by several markers such as Hep Par 1, polyclonal CEA, CD10, and the recently developed arginase (Arg-1), but they cannot distinguish HCC from benign hepatocytes. Hep Par 1 has a diffuse cytoplasmic granular staining pattern in normal and neoplastic hepatocytes[37-40]. In HCC, staining with polyclonal CEA and CD10 produces a canalicular staining pattern that has been attributed to cross reactivity with the biliary glycoprotein on the canalicular surface. The canalicular staining pattern is specific for HCC and is not seen in cholangiocarcinoma and metastatic adenocarcinoma, but its sensitivity has been variably reported, ranging from 50%-96%[41-45]. Arg-1 is a manganese metalloenzyme active in the urea cycle that is a recently developed immunohistochemical marker of hepatocellular neoplasms of high sensitivity and specificity when used alone or in combination with glypican-3 or Hep Par 1[46-48].

The differential diagnosis of HCC from other hepatocytic lesions includes hepatocellular adenoma, focal nodular hyperplasia, and dysplastic and macroregenerative nodules, especially in well-differential HCC. Other malignant tumors that can cause diagnostic difficulties include cholangiocarcinoma and metastatic tumors including carcinoma from any sites and melanomas. These can be differentiated with clinical history, radiological findings, histomorphology, reticulin stain and immunohistochemical markers. Small samples of biopsy tissue material may cause diagnostic challenges.

Fibrolamellar variant of HCC (fibrolamellar carcinoma) consists approximately 0.5%-1% of all HCC. It has a unique clinical presentation, pathological feature, and biology than the typical HCC. It tends to occur in late teenage years and young adults years. Unlike the typical HCC that often arises in a background of chronic liver disease or cirrhosis, fibrolamellar carcinoma typically arises in liver without any underlying liver diseases. 60%-70% of fibrolamellar carcinomas have a central scar, which appear as thick lamellar bands of fibrosis under microscopy as one of the most characteristic low power feature (Figure 2A). The tumor cells are large and polygonal with abundant eosinophilic cytoplasm, large vesiculated nuclei, and large nucleoli (Figure 2B)[49].

For fear of needle tract seeding and risk of bleeding, HCC with the classic contrast enhanced imaging appearance, *i.e.,* arterial enhancement with portal and delayed venous washout typically do not require preoperative tissue confirmation by core needle biopsy or fine needle aspiration biopsy, however, in equivocal cases, which are not uncommon, histopathology remains central in the diagnosis of HCC.

MEDICAL IMAGING OF HCC

Medical imaging has been an essential resource for the detection and management of HCC. The appropriate use of the different imaging modalities allows optimization of resources and more accurate results. Screening, characterization, staging, therapeutic interventions and response to treatment assessment are some of the most important uses of imaging studies.

Ultrasound

Ultrasound (US) imaging utilizes high frequency sound waves to generate images of the tissues. Most commonly it does not involve the use of intravenous (*iv*) contrast or radiation and therefore there are no contraindications for its use. It is also one of the least costly imaging modalities. These facts make it the exam of choice for screening of HCC in high-risk population[50,51] (Figure 3). Another application of US in HCC patients is to guide procedures including biopsies, radiofrequency ablation and ethanol injection of tumors.

The limitations of US are the low specificity for characterization of liver masses, thus it is frequently necessary to follow up the patients with contrasted computed tomography (CT) or magnetic resonance imaging (MRI) for confirmation of the diagnosis. Recent studies have demonstrated that the use of *iv* contrast for US increases the accuracy for tumor characterization of this modality, making it comparable to contrast enhanced CT or MRI[52]. US contrast is made of microscopic

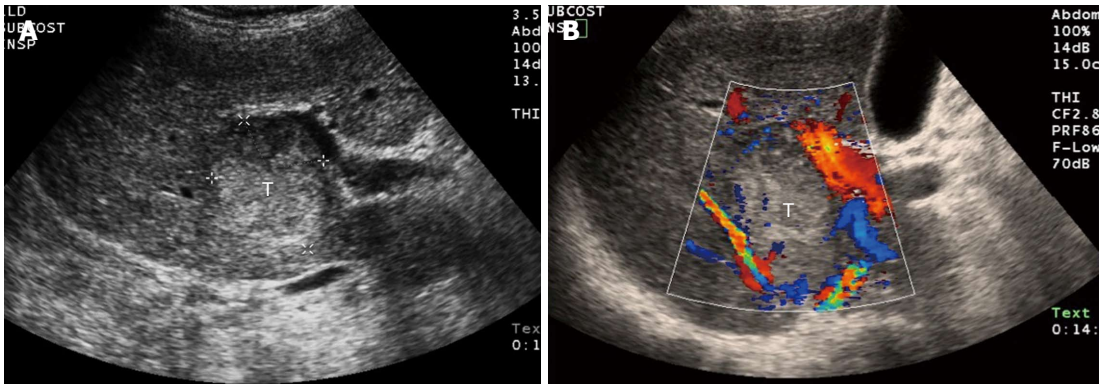

Figure 3 A 68-year-old male with cirrhosis and surgically proven hepatocellular carcinoma. A: Thirty-four seconds after intravenous injection of ultrasound contrast (microbubbles) there is tumor (T with dashed line) enhancement; B: One and half minutes after injection the tumor (T with dashed line) is washing out of contrast. The images on the right side are a conventional sonogram (non-contrasted) of the lesion. The image on the left is a pulse inversion harmonics ultrasound for better visualization of ultrasound contrast media.

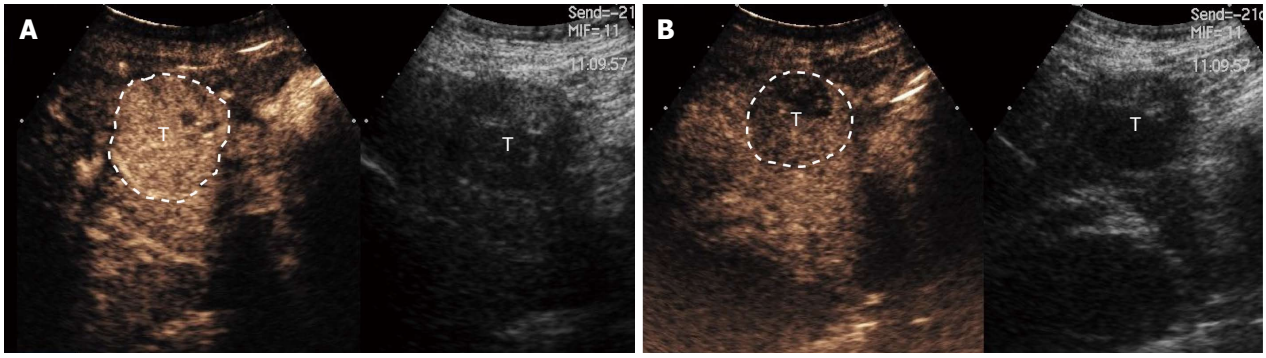

Figure 4 Ultrasound of hepatocellular carcinoma. A: Ultrasound of the liver demonstrates a heterogeneous tumor (T) in the right lobe of the liver that was later characterized as definite hepatocellular carcinoma by computed tomography; B: Same lesion (T) using color Doppler ultrasound images to demonstrate blood flow in the adjacent vessels.

encapsulated gas bubbles. Although the Federal Drug Administration has not approved the use of US contrast for abdominal imaging in the United States it is routinely used for liver mass characterization in Europe, Canada and Asia. US contrasted studies show similar enhancing characteristics than other tomographic contrasted imaging modalities like CT or MRI (Figure 4).

New advances in US technology include the use of shear wave elastography[53,54]. This new technique uses estimations of the velocity of the sound in the tissue for quantitative assessment of fibrosis and prediction of the risk of HCC development. Shear wave elastography can also be used for characterization of liver tumors including HCC[55], and also for assessment of response to treatment[56].

CT

CT is the workhorse of medical imaging for diagnosis and staging of HCC. It utilizes measurements of the attenuation of X-rays to generate images. For accurate detection and diagnosis of HCC, the correct use of iodinated *iv* contrast, specifically with high injection rates and multiphase imaging with accurate timing for each phase (late arterial, portal venous and delayed) is extremely important. The almost exclusive arterial blood supply of HCC determines earlier arrival of injected *iv* contrast, compared to liver parenchyma mainly supplied by the portal vein. This early enhancement of HCC in contrasted studies is best captured in the set of images of arterial phase; later on, the HCC typically washes-out of contrast earlier than the liver parenchyma, best demonstrated in the 3-5 min delayed set of images. Also the tumor capsule shows characteristic enhancement in the delayed phase due to retention of contrast within the fibrous tissue of the capsule, as shown in Figure 5. Therefore a tumoral mass enhancing in the arterial phase, and washing out on the delayed phase in a high risk patient is a very specific finding for the diagnosis of HCC with a positive predictive value (PPV) of 98.8% for cirrhotic patients[57], and therefore allows the medical team to treat the patient without the need of a diagnostic biopsy.

CT is commonly used for staging HCC, with excellent detection of vascular invasion and metastasis (Figure 6). CT can also demonstrate the presence of intratumoral calcifications, which sometimes can support the diagnosis of HCC.

Potential future advances in CT imaging of HCC include standard use of perfusion analysis and dual-energy imaging for assessment of response to therapy,

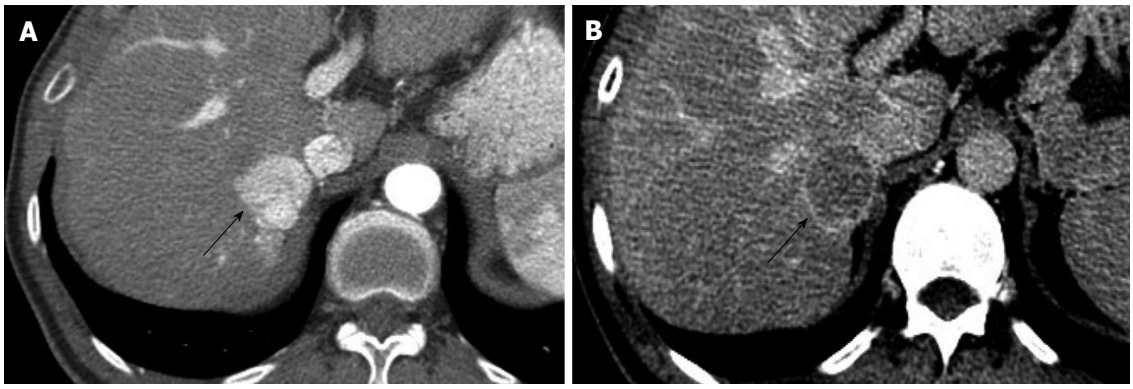

Figure 5 Computed tomography of hepatocellular carcinoma in 48-year-old male with hepatitis C. A: Arterial phase contrast enhanced CT of the liver shows a strongly enhancing mass (arrow) in the right lobe, adjacent to the IVC. B: The same lesion (arrow) washes-out of contrast on the delayed phase and shows a thin capsule, this is diagnostic for HCC and corresponds to LI-RADS category 5. HCC: Hepatocellular carcinoma; CT: Computed tomography; LI-RADS: Liver imaging reporting and data system; IVC: Inferior vena cava.

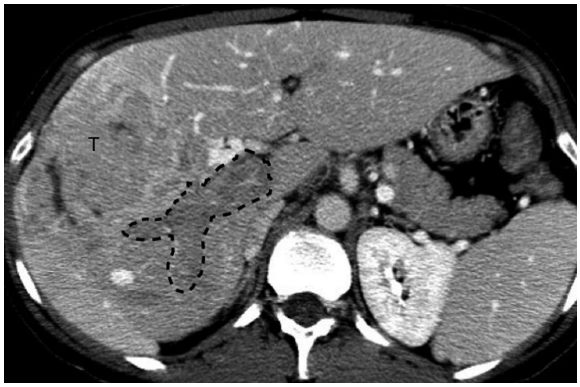

Figure 6 Portal vein invasion by hepatocellular carcinoma. Computed tomography in portal venous phase shows a right lobe mass (T) and lack of enhancement of the portal vein (outlined), consistent with tumor invasion.

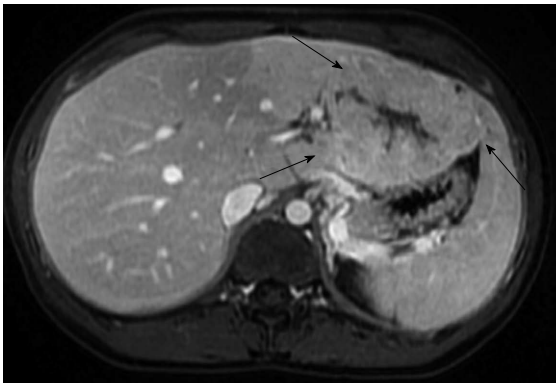

Figure 7 Magnetic resonance imaging of hepatocellular carcinoma in 19-year-old female. Post contrast liver magnetic resonance imaging in portal venous phase shows a large mass (arrows) arising from the left lobe of a liver without cirrhosis. This lesion that has some imaging similarities with focal nodular hyperplasia, corresponded to fibrolamellar carcinoma on pathologic analysis.

with some studies showing changes in arterial perfusion (associated with improve in survival) earlier than changes in tumor diameter[58,59] Potential contraindications for CT include: anaphylaxis to iodinated contrast media, severe renal failure, and pregnancy.

MAGNETIC RESONANCE

MR generates medical images utilizing radiofrequency pulses and changes in magnetic gradients within a very strong magnetic field. Similar to CT, it is crucial to use *iv* contrast for detection and characterization of liver masses, as shown in Figure 7. In the case of MR, the contrast contains Gadolinium, a strong paramagnetic element that causes the surrounding molecules to release energy and show increased tissue intensity (enhancement). Injection rate and timing of the multiphase post-contrast images are also crucial for accuracy of the test. As with other modalities radiologists look for enhancement of the mass in arterial phase, and washout with capsular enhancement in delayed phase to make the diagnosis of HCC[60-63]. In addition, MR can demonstrate the presence of ancillary findings including tumoral fat, hemorrhage and increased signal in non-contrasted images. Contraindications include renal failure, first trimester pregnancy and pacemakers. Perfusion analysis and diffusion weighted imaging (DWI) to evaluate response to local therapy demonstrating enhancement changes earlier than size response. Whole body DWI for detection of metastatic disease have been used mostly in research setting but the standardization of protocols and other advances will allow the wide use of these techniques in the common clinical setting.

Angiography

Angiography is not used as a diagnostic tool anymore but has become a key therapeutic tool for HCC when used to deliver treatment in Trans Arterial Chemo-Embolization (TACE) and radioembolization with yttrium-90 (Y90) in patients with non-resectable tumors.

Positron emission tomography

Fluorodeoxyglucose (FDG) positron emission tomography (PET) and FDG CT-PET studies have low sensitivity for well-differentiated HCC and therefore are not commonly used to diagnose or stage the disease[64,65]. Nevertheless it could be of some value in cases of poorly differentiated

Table 2 Liver imaging reporting and data system categories

LI-RADS category	Significance
1	Definitely benign
2	Probably benign
3	Indeterminate
4	Probably HCC
5	Definitely HCC

LI-RADS: Liver imaging reporting and data system; HCC: Hepatocellular carcinoma.

tumors to identify metastasis. The development of new PET radiotracers for HCC could potentially increase the use for diagnosis, staging and assessment of response to therapy.

Accuracy of different imaging modalities for HCC diagnosis

Comparison between modalities like US, CT and MR is difficult due to differences in the methodology of the multiple published studies[60-63], but a systematic review by Colli *et al*[66] showed a sensitivity of 60% (95%CI: 44-76) and specificity of 97% (95%CI: 95-98) for US; for CT, the sensitivity was 68% (95%CI: 55-80) and specificity was 93% (95%CI: 89-96). The sensitivity for MR was 81% (95%CI: 70-91) and specificity was 85% (95%CI: 77-93). Accuracy of imaging tests correlates directly with tumor size with sensitivities and specificities around 30% for < 1 cm lesions and more than 90% for lesions > 2 cm[67].

Liver imaging reporting and data system

The American College of Radiology has directed an effort to standardize the reporting and data collection of CT and MRI for HCC in cirrhotic population, developing the liver imaging reporting and data system (LI-RADS) classification of lesions (http://www.acr.org/Quality-Safety/Resources/LIRADS). LI-RADS divide the lesions in 5 categories from benign (category1-2) to definitely HCC (category 5). The latest version of LI-RADS is now concordant with the United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Network classification, making it valuable for health care workers involved in the liver transplant teams[68]. The PPV for CT and MR in the category 5 lesions is so high that the patient does not require a biopsy to be treated. Table 2, shows the list of categories with the significance of each. Category 5 lesions can be treated without the need of histology confirmation. This classification should be applied only in cirrhotic patients.

SURGICAL RESECTION OF HCC

Liver resection (LR), also known as partial hepatectomy (PH) is a potentially curative surgical treatment option for patients with HCC, and is feasible in approximately 15% to 20% of all case presentations. The goal of LR is to remove the HCC with an adequate margin, while preserving as much functional liver parenchyma with minimal blood loss and no complications. The safety, result and outcomes of PH for HCC and cirrhotic patients have improved substantially over the last three decades. This has to be attributed to refined patient evaluation and selection, the ability to manipulate the future liver remnant volume, advances in surgical and anesthetic techniques, and the enhanced peri-operative management of these patients. The operative mortality for LR is less than 5% even in cirrhotic patients or those undergoing major PH, and the 5-year overall survival is over 50% for HCC[69-71].

Principles of LR for HCC

Patient selection: Patient selection for LR in HCC is unique in that in addition to the standard assessment of the patient's ability to tolerate the procedure, anesthetic, potential complications, and the biology and stage of the cancer, the synthetic function of the liver parenchyma and the presence of portal hypertension must also be accounted for, as most patients will have some degree of fibrosis or cirrhosis, which will determine the liver's capacity to regenerate and recover function following PH[72].

A LR patient must be medically fit for a major operation, have no significant medical co-morbidities, and should have a good Eastern Cooperative Oncology Group (ECOG) performance status and quality of life score. Although there is no strict cut-off in terms of chronological age, patients with advanced age (> 70) will have limited physiologic capacity for liver regeneration, which must be accounted for in the surgical planning[73].

Adequacy of hepatic reserve in the future liver remnant (FLR) is most commonly assessed using the Child-Turcotte-Pugh (CTP) score, where CTP A5 through B7 patients are considered reasonable candidates for LR. Pre-operative Model for End-stage Liver Disease (MELD) score of greater than 9 predicts increased operative mortality for major PHs and can supplement the CTP score[74].

The presence of portal hypertension is a relative contraindication to PH, where only select minor PHs are appropriate, and trans-jugular intra-hepatic porto-systemic gradient (PSG) measurements (significant portal hypertension when PSG measurements are greater than 10 mmHg) can help to elucidate equivocal cases[75]. Volumetric measurement using CT/MRI is important in planning major resections and in patients with cirrhosis. Although up to 80% of functional liver can be resected safely if two contiguous healthy liver segments are preserved, increased FLRs [FLR% = FLR/(total liver volume-tumor volume)] are necessary for fibrotic (> 30%) and cirrhotic (> 40%) livers. Preoperative portal vein embolization (PVE) is indicated in patients with small FLRs ($\leqslant$ 20% in normal and $\leqslant$ 40% in fibrotic/cirrhotic liver), and a FLR volume increase > 5% with PVE predicts low risk of post-PH liver failure[76,77].

Biologic markers to predict HCC tumor biology are

under development. Current surrogate prognostic factors, such as the stage and extent of the HCC used to formulate the operative plan is based on tumor size, number, and vascular invasion evaluated by multiphase liver protocol CT or MRI. For primary HCC tumors, Ho *et al*[78] reported that larger tumor sizes and AFP levels over 400 ng/mL were associated with postresection recurrence of HCC, exceeding the University of California at San Francisco (UCSF) criteria. Other markers such as retinoic acid-induced protein 3 as well as miRNA expression profiles could be used to predict poor prognosis to assess the risk of disease recurrence after liver transplantion[79,80].

Large tumor size has traditionally been a relative contraindication to LR given the elevated risk of vascular invasion. However, as many HCCs over 10 cm in size that do not invade the vasculature are amenable to PH with good results, identification of such tumors is important. Surgical techniques such as the anterior hanging maneuver can be employed to facilitate resection of such large HCCs[81-83].

Similarly, multifocal disease generally increases the risk of recurrence and is a relative contraindication to LR. However, select patients with multifocal HCC outside of the Milan criteria for orthotopic liver transplantation (OLT) can be offered PH in combination with ablative and catheter-directed therapies in the absence of vascular invasion or HCV as the etiology for cirrhosis. Routine use of TACE as a neo-adjuvant therapy has not been demonstrated beneficial. Y90 radio-embolization prior to LR may play a role in down-staging tumors and is being investigated[84,85].

LR for HCC invading the portal vein or hepatic veins remains controversial, as the outcomes have been disappointing. However, highly selected cases of HCCs with tumor thrombus not extending into the major vascular trunks, *e.g.,* main portal or hepatic veins, can be resected with reasonable outcomes[86,87].

Ruptured HCC is a life-threatening condition occurring in approximately 4.5% to 14.5% of cases, and carries a grim prognosis. Control of bleeding is best accomplished using hepatic artery embolization. Surgical ligation of the hepatic artery with packing, plication or selective resection of the bleeding tumor can be considered in refractory cases. Interval PH can be considered in select cases where laparoscopy has ruled out peritoneal carcinomatosis, and can provide long-term survival in highly selected cases[88].

Technical considerations: What is considered an adequate width for the surgical resection margin has been a controversial topic of debate. The only randomized controlled trial evaluating the influence of the width of resection margin for HCC concluded that the recurrence rates decreased and 3- and 5-year survival rates increased when aiming for 2 cm margins compared with 1 cm margins. However, the meta-analysis of this and four non-randomized trials demonstrate no significant difference in recurrence rate, or 1-, 3-, and 5-year survival rates between resection margin < 1 cm and margin > 1 cm[89].

The liver consists of eight Couinaud segments with distinct vascular inflow/outflow and biliary drainage. Segment-based anatomical PH to remove all intrasegmental portal vein branches is not only less bloody given the ability to gain control of the inflow to the segment(s) and parenchymal division through relatively vessel-free regions, but has also shown to provide better 5-year overall and disease-free survival rates. This is presumably due to removal of microscopic tumor foci and is recommended when feasible. However, non-anatomical PH is oftentimes necessary in an effort to preserve as much FLR as possible in cirrhotic patients[90].

Hemorrhage is the most significant operative risk for LR especially for cirrhotic patients, and excessive bleeding is an independent risk factor for cancer recurrence and poor survival. Several surgical and anesthetic maneuvers have been developed to minimize intra-operative hemorrhage.

Low central venous pressure (CVP) anesthesia is preferred when feasible to minimize hemorrhage from the hepatic veins and inferior vena cava. Low CVP is maintained by *iv* fluid restriction and administration of diuretics and/or vasodilators. For open PHs, the patient is placed in Trendelenberg position to increase preload and cardiac output for better end-organ perfusion. For laparoscopic procedures, the patient is placed in reverse Trendelenberg position. Intermittent occlusion of the vascular inflow, or the Pringle maneuver with ischemic preconditioning, is selectively utilized for challenging parenchymal transections where potential massive hemorrhage is a concern[91].

Parenchymal division can be performed in a variety of ways, *e.g.,* Clamp-crush technique, cavitron ultrasonic surgical aspirator, and Erbe Hydro-jet clear away the liver cells allowing for visualization of the vascular and biliary tributaries for ligation of these structures, whereas Harmonic scalpel, Sonocision, LigaSureand TissueLinkdissecting sealer are high-energy devices that can simultaneously seal blood vessels and transect liver tissue. No major difference in blood loss, morbidity or mortality has been demonstrated between these techniques, and choice is best left to the circumstances of the resection and surgeon preference and comfort level[92].

Comparison to other "Curative" modalities: Comparison of these two modalities is quite challenging given that PH and OLT have overlapping yet differing patient selection criteria. Meta-analyses have demonstrated that OLT increased late disease-free and overall survival rates when compared to PH. The benefit of OLT is offset by the higher short-term mortality, shortage of donor organ availability, and long transplant wait times associated with more patient deaths[93].

PH as bridge to salvage OLT, especially with the increasing number of patients with non-alcoholic fatty liver disease associated HCC where cirrhosis is not a

mandatory step to development of HCC, and the advent of minimally invasive approaches to PH, is feasible and can be used to partially address the donor shortage issue. However, given current UNOS policies regarding retraction of tumor exception points when a solitary HCC is resected, a careful balance must be practiced with the patient's interest in mind.

Multiple systematic reviews of randomized and non-randomized trials comparing PH to radio-frequency ablation (RFA) for patients with HCC meeting Milan criteria, *i.e.,* small tumors, few in number, demonstrate that while PH afforded better long-term, *i.e.,* 3- and 5-year disease-free and overall survival over RFA, it came at a cost of higher rates of complications and longer hospital stays. Cirrhotic patients with HCC tumor size less than 3 cm and three or less tumor numbers should be carefully evaluated and selected for either modality based on patient and tumor characteristics[94].

Laparoscopic surgical resection: The first laparoscopic PH for malignant disease was reported just over two decades ago. However, the last decade was met with an explosion in the number of reported cases totaling over 3000, and as more experience has accrued, this growth has been especially true in the treatment of HCC. It is well established that post-operative morbidity and longer-term complications such as incisional hernias are lower in laparoscopic PH compared to the open approach. Furthermore, liver-specific complications in cirrhotic patients are lower in the laparoscopic group, thought to be due in part to less severance of collateral vessels in the abdominal wall. Laparoscopic PH possesses advantages over the open approach in minimizing blood loss. With better visualization *via* 6- to 10-time, high-definition magnification, allowing for improved tissue handling and control of vessels, especially with the robotic approach which affords added dexterity, and a relative tamponade effect on the hepatic veins provided by the pneumoperitoneum, blood loss and transfusion requirements have been shown to be more favorable for the laparoscopic cohort. In an era of cost containment, the financial aspects are playing an increasingly important role. Studies directly comparing laparoscopic *vs* open PHs demonstrate that the total hospital costs for laparoscopic PHs are equivalent or less than those of open cases. The increased operating room costs are offset by the shorter length of stay following laparoscopic PH. Studies report equivalent to better margin status, recurrence rates, and overall survival figures for patients with HCC. Furthermore, significant decreases in operating room time, blood loss, transfusion and technical difficulty of salvage transplantations following laparoscopic PH for HCC have been reported. Also, there is emerging evidence that laparoscopic procedures may lessen the acute metabolic stress response accompanied by a transient state of post-operative immunosuppression, which may impact oncologic outcomes[95,96].

LIVER TRANSPLANTATION FOR HCC

Liver transplantation for HCC in the early years of transplantation was complicated by high rates of cancer recurrence and poor 5-year survival. In the late 1990s, there was emerging data suggesting that limiting transplant candidacy based on tumor characteristics could result in good outcomes, comparable to non-HCC patients. The hallmark study of Mazzaferro *et al*[96] in 1996 described 75% 4-year survival in a cohort of patients with HCC limited to a single tumor $\leqslant$ 5 cm or up to 3 tumors none greater than 3 cm[97]. These criteria, now known as the Milan Criteria, have become the standard for patient selection. In the United States, patients with HCC within Milan Criteria have been assigned priority with standardized exception points. When first created, this exception originally granted 29 MELD points, but was decreased to 24 points in 2003 and then 22 points in 2005, due to concerns that HCC patients were receiving excessive priority. There remains concern by some in the transplant community that HCC patients continue to have excess priority. With the increasing prevalence of HCC and the priority given to HCC for liver transplantation, the proportion of patients transplanted in the US with an HCC exception now exceeds 25% of total liver transplant volumes.

As patients with HCC await liver transplantation, there is a significant risk of tumor progression beyond transplant criteria, resulting in list drop out and exclusion from transplant. This risk can exceed 30% at one year for those with tumors > 3 cm[98]. Concern for tumor progression has resulted in the frequent use of locoregional tumor treatment as bridging therapy for those awaiting transplant. Many centers pursue locoregional therapy for patients who are likely to be on the waitlist for more than 6 mo prior to being transplanted. Modalities used to treat tumors prior to transplant include RFA, microwave ablation, TACE, transarterial radioembolization, percutaneous ethanol injection and irreversible electroporation. The impact of pre-transplant tumor treatment is not well understood, as studies have shown conflicting results. It appears that bridging therapy reduces the risk of list drop out, improving the likelihood that listed HCC patients will undergo transplant[99,100]. It is unclear if pre-transplant bridging therapy has any impact on post-transplant outcomes. Studies have been retrospective, and confounded by the fact that good response to locoregional therapy is likely a marker of favorable tumor biology. A study by Yao *et al*[100] showed an improvement in post-transplant survival in those who received bridging therapy; however, multiple additional studies show no impact on post-transplant survival[101-103].

Milan criteria remains the most commonly utilized inclusion criteria for liver transplantation, yet several other guidelines have been proposed and are being used by various centers around the globe. The rationale for more liberal tumor criteria is the concern that Milan Criteria may be too restrictive and exclude patients who

could benefit from transplant with an acceptable risk of HCC recurrence. There are numerous criteria that have been proposed, including Up-To-Seven, UCSF, Toronto, Asan, CUN and Kyoto[104-108]. The best described of these include the Up-To-Seven criteria, in which the sum of the number of tumors and the diameter of the largest tumor (in cm) does not exceed 7. The UCSF criteria allow for a single tumor up to 6.5 cm, or up to three tumors none greater than 4.5 cm with a total tumor volume of less than 8 cm, with no extra hepatic disease or macrovascular invasion. The most recent published data report excellent 1- and 5-year survival of 90% and 75% for patients with HCC within UCSF criteria[105].

A similar concept is that of downstaging, in which patients with HCC beyond Milan Criteria undergo locoregional tumor treatment, and those with reduction in tumor burden within Milan Criteria are eligible for transplantation. This concept was first introduced in 1997 by Majno *et al*[108], noting improved post-transplant survival in those who responded to TACE with a reduction in tumor burden to meet Milan Criteria[109]. Multiple centers and UNOS Regions currently have proposed downstaging criteria, including the UCSF group, which has published their outcomes. UCSF downstaging criteria allows for initial tumor burden to include 1 lesion > 5 cm and ⩽ 8 cm, 2 or 3 lesions each ⩽ 5 cm with total tumor diameter ⩽ 8 cm, 4 or 5 lesions none > 3 cm with total tumor diameter ⩽ 8 cm, and no vascular invasion on imaging. Importantly, this algorithm requires 3 mo of imaging stability following downstaging to Milan prior to listing, to allow for observation of tumor biology. Five year patient survival in this cohort is excellent, at 80%[110].

In addition to tumor size and number, multiple additional factors have emerged as potential predictors of HCC recurrence following liver transplantation. The presence of vascular invasion on explant pathology is one of the strongest predictors of recurrence. Histologic grade of tumor differentiation has also been shown in multiple studies to be associated with the risk of tumor recurrence, with well-differentiated tumors having lower risk, and poorly-differentiated tumors being at high risk. The predictive value of pre-transplant alphafetoprotein (AFP) has been highlighted in multiple studies, with a strong association of AFP > 400 and AFP > 1000 with increased risk of post-transplant HCC recurrence[111]. As increased knowledge regarding molecular markers of HCC is gathered, certain microRNA sequences have been identified which can help predict tumor biology, including post-transplant recurrence[112]. It is possible that such biomarkers will help with selection of HCC patients for transplant in the future.

Once transplanted, the use of various immunosuppressive medications may impact the risk of cancer recurrence in HCC patients. While the data regarding the impact of steroids, calcineurin inhibitors and induction agents is highly variable, there is compelling data regarding the effects of the mTOR inhibitor sirolimus. Several studies, including a meta-analysis, have outlined a reduction in HCC recurrence and improved post-transplant survival for HCC patients receiving sirolimus[113,114]. If HCC does recur after transplant, surgical resection of isolated recurrences is often pursued. The use of sorafenib post-transplant has been reported in multiple studies, with mixed results regarding tolerability and efficacy[115,116].

LOCOREGIONAL THERAPY

When determining the most appropriate treatment for HCC, the patient's underlying liver function and performance status play pivotal roles[117]. For patients who are not considered surgically resectable but otherwise may be treatment candidates, types of therapy include ablation and arterial embolization.

Ablation for HCC

Several ablation techniques have been used to treat HCC. RFA, microwave ablation, percutaneous ethanol injection, cryoablation, and irreversible electroporation are the most common modalities. For the purposes of this review, RFA will be discussed since that is the most common ablative technology used with the strongest evidence.

RFA is a thermal-based ablative technology, using energy to induce local coagulative necrosis. *Via* an alternating current, surrounding tissue heats from ion movement and friction. Tissue temperature in excess of 60 degrees Celsius induces local coagulative necrosis. RFA can be either done *via* a percutaneous route, laparascopic route, or *via* open surgery. It is often performed using real-time US guidance, in order to appropriately position the probe and to monitor the area of ablation (Figure 8). Numerous series have demonstrated consistently highly local tumor control rates, with relatively low rates of local tumor recurrence. Many have considered RFA to be near-equivalent to surgical resection for tumors < 3 cm, with similar 5-year survival rates[118,119]. While local tumor recurrence continues to be an issue with RFA, the recurrence rates are favorable compared to percutaneous ethanol injection[120], and have improved over time as new ablation devices and better imaging guidance have been utilized. Overall, there is a wide range of reported results both in terms of local tumor recurrence rates and overall survival. This is likely due to wide-ranging patient selection and varying levels of operator expertise. While several factors can affect local tumor recurrence rates, tumor size has been shown to be the most significant factor[121].

While RFA has been used extensively in various types of tumors, several drawbacks remain. Thermal damage to adjacent non-target structures can result in significant complications. Additionally, ablation of tissue adjacent to flowing blood is affected by a "heat sink", whereby sub-optimal temperatures are reached, resulting in incomplete ablation[122]. Due to these limitations, the appropriate use of RFA is often location dependent. Finally, several liver transplant centers consider RFA to be a relative contraindication for patients undergoing

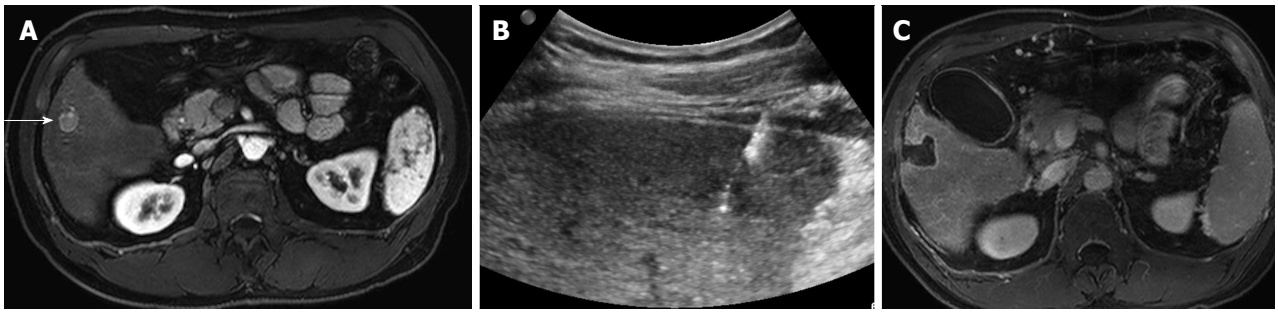

Figure 8 Radiofrequency ablation of a focal hepatocellular carcinoma. A: Contrast-enhanced MR of a 60-year-old male with cirrhosis demonstrates a single hepatocellular carcinoma in the right hepatic lobe (arrow); B: Ultrasound demonstrates a radiofrequency probe coursing through the hypoechoic tumor; C: Contrast-enhanced MR 1 mo after radio-frequency ablation demonstrates a large ablation defect, without any residual enhancement to suggest viable tumor. MR: Magnetic resonance.

liver transplantation, due to the potential risk of "tract seeding"[123].

Embolization for HCC

Patients with HCC frequently present in later stages when curative treatments are no longer an option[124]. Therefore, the majority of patients with HCC eligible for treatment will undergo non-curative treatments, of which arterial chemoembolization is the most commonly performed procedure. As stated above in the section of surgical treatment, arterial embolization takes advantage of HCC's reliance on the hepatic artery as its sole blood supply, as opposed to the portal vein. By utilizing hepatic arterial flow, therapeutics can be delivered in a selective manner to hypervascular tumors such as HCC. The mechanism of cell death may be from several causes depending on the embolic used. Tumor necrosis can result from tissue ischemia, a chemotherapeutic effect, or *via* internal radiation. By performing selective embolization (*i.e.,* lobar or segmental), the degree of hepatic tissue exposed to the embolic agent can be potentially minimized and therefore complications and effectiveness can be optimized. Trans-arterial chemoembolization and Y90 radioembolization are the two most commonly used embolic procedures for HCC, and they will be discussed further.

Chemoembolization

Chemoembolization is defined as the infusion of a mixture of chemotherapeutic agents with or without ethiodized oil followed by embolization with particles such as polyvinyl alcohol, calibrated microspheres, or gelfoam[125]. This is performed by obtaining arterial access *via* the femoral artery. A microcatheter is then advanced into the hepatic artery, and typically advanced further into the vessel supplying the tumor.

Depending on the techniques employed, tumor death is caused by the cytotoxic effects through achieving high intra-tumoral concentration of chemotherapy, the ischemia induced by embolization, or both. In the case of oil-based chemoembolization, the mechanism of action relates to both the cytotoxic effects of the chemotherapy and ischemic effects induced by embolization. Embolization also prevents washout of the chemotherapeutic agent into the systemic circulation. Embolization with drug-eluting beads has gained popularity over traditional oil-based chemoembolization. Due to the prolonged binding properties of drug-eluting beads with doxorubicin, the drug is slowly released into the tumor reaching higher local concentration and decreased systemic concentration when compared to oil-based chemoembolization. This may allow for decreased side effects and improved tolerance in some patients[126].

Variation in patient selection and procedure technique among institutions has led to significant heterogeneity in response and survival. The publication of two randomized trials in 2002 established chemoembolization as standard of care for patients with unresectable HCC[127,128]. Llovet *et al*[127] reported survival probabilities at 1 year and 2 years, which were 75% and 50% for embolization, 82% and 63% for chemoembolization, and 63% and 27% for control (chemoembolization *vs* control, *i.e.,* best supportive care, not tumor treatment, $P = 0.009$). The ensuing widespread use and proven results of chemoembolization have resulted in its incorporation into standard treatment guidelines for HCC[129,130].

Chemoembolization is generally considered the first line non-curative therapy for patients with early- and intermediate-stage HCC. Chemoembolization can also be used as a "bridge to transplant". In these patients the procedure is performed to prevent disease progression beyond Milan criteria (single tumor $\leqslant$ 5 or three tumors $\leqslant$ 3 cm). Patients undergoing chemoembolization should have adequate hepatic function (Child-Pugh Class A or B) and acceptable functional performance status (ECOG 0-2). Chemoembolization can also be done in conjunction with an ablation procedure in intermediate-sized HCC (3-5 cm)[131].

Post-embolization syndrome is the most common side effect of chemoembolization. This is a constellation of symptoms including low-grade fever, abdominal pain, nausea, and ileus, often occurring 48-72 h following the procedure. Serious toxicities from chemoembolization include liver failure, biloma, and abscess formation.

Radioembolization

Y90 radioembolization is a relatively newer embolic

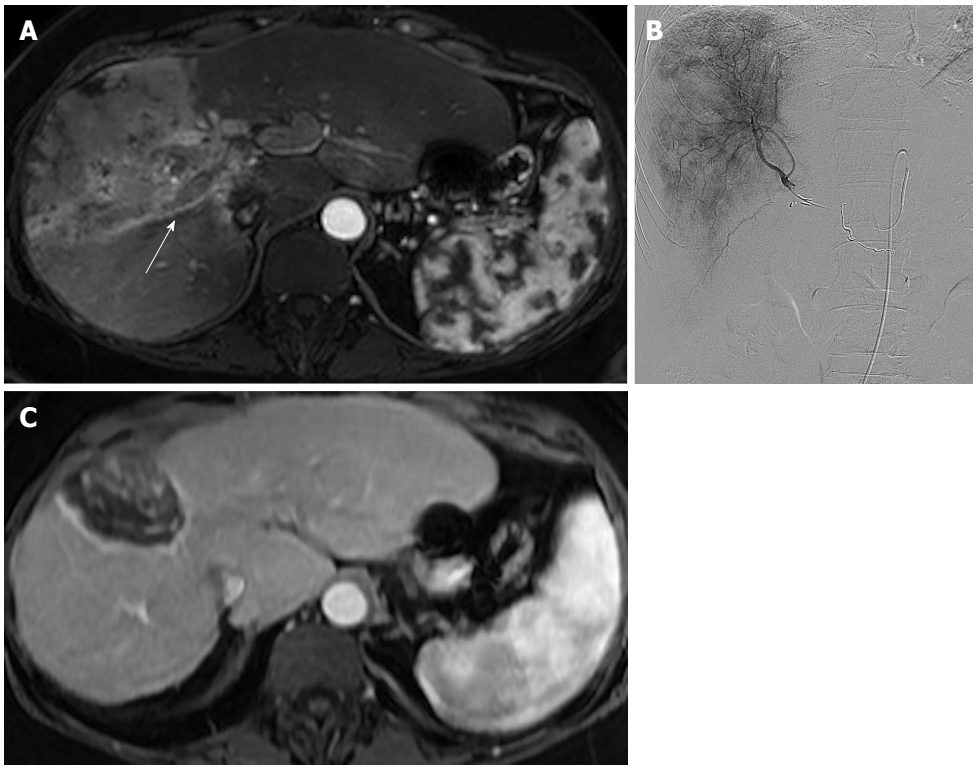

Figure 9 Yttrium-90 radioembolization of diffuse, infiltrative hepatocellular carcinoma with vascular invasion. A: Contrast-enhanced MR of a 55-year-old female with cirrhosis demonstrates an infiltrative hepatocellular carcinoma replacing the anterior right hepatic lobe. Tumor-associated portal venous thrombus is present in the right portal vein (arrow); B: Digital subtraction angiogram with a microcatheter in the right hepatic artery shows diffuse tumor hypervascularity. The patient underwent a right hepatic artery Y90 radioembolization; C: Contrast-enhanced MR 12 mo after radioembolization demonstrates complete necrosis of the entire tumor with marked reduction in size. MR: Magnetic resonance; Y90: Yttrium-90.

procedure compared to chemoembolization. Many consider it to not be a true embolization procedure since the particle size is much smaller than those used in chemoembolization. Therefore, arterial stasis does not typically occur during radioembolization. Y90 is a beta emitter, with the radioactive element either embedded within or surface-coated on the particles. The average tissue penetration of beta radiation in this case is 2.5 mm, with a maximum distance of 11 mm. Y90 particles use internal radioactivity as its mechanism of action. Since HCC is hypervascular, preferential flow of microembolic particles to the tumor potentially increases dose to the tumor compared to the surround hepatic parenchyma. Using these principles, hypervascular tumors can receive lethal doses of radiation while the surrounding tissue is relatively spared (Figure 9).

Radioembolization is a complex multi-step procedure[132]. The first is a mapping angiogram, where a diagnostic angiogram is performed in order to elucidate the hepatic arterial anatomy. During this initial step, extrahepatic vessels (*i.e.,* gastroduodenal artery, right gastric artery) may be prophylactically coil embolized in order to prevent inadvertent non-target deposition of Y90 microspheres into the gut. At the conclusion of the mapping angiogram, a small dose of Tc99m-MAA is administered into the hepatic artery and a nuclear medicine scan is performed in order to determine the degree of shunting into the lungs, and to assess for the potential of extrahepatic uptake. Patients undergo a second angiogram 2-14 d later, and during this angiogram the Y90 infusion is performed.

Several studies have now reported tumor response rates, toxicity, and survival in patients undergoing Y90 radioembolization[133-135]. Salem *et al*[131] reported a prospective evaluation of 291 patients, where patients were stratified based on their tumor burden and degree of liver dysfunction. Patients with Child-Pugh A cirrhosis had significantly longer lengths of survival compared to those with advance liver dysfunction. Many series have also demonstrated the effectives of Y90 radioembolization in the setting of portal vein thrombosis[136]. These patients present a particular challenge given that blood supply to the liver depends primarily on the hepatic artery and portal flow is compromised by the obstructive tumor. If hepatic arterial vessels are embolized (as with chemoembolization) in order to treat advancing disease, blood flow to the liver is further compromised, increasing the risk of liver failure. Thanks to its minimally embolic properties, radioembolization can be used in these patients without compromising the hepatic arterial flow, preserving the functional liver reserve.

Fatigue is the most frequent observed toxicity. Abdominal pain and nausea can also occur, but typically at a lower incidence and severity compared to chemoembolization[137]. Serious complications include non-target embolization, which can result in gastrointestinal

ulceration or cholecystitis. With increasing operator experience, the incidence of ulcers can be minimized. Finally, radiation induced liver disease occurs in 2%-20% of patients. In rare cases, this can be associated with progressive liver failure.

EXTERNAL BEAM RADIATION THERAPY IN HCC

Although HCC is considered a radioresponsive tumor, the role of external beam radiation therapy (EBRT) has historically been limited in the treatment of HCC due to the high radiosensitivity of normal liver tissue and the risk of radiation-induced liver disease (RILD) with whole liver RT[138]. RILD typically occur within 3-4 mo after EBRT and may progress to permanent liver failure and death with no established effective treatment other than supportive care. In general, the risk of RILD is considered to be primarily related to the volume of normal liver that is exposed to potentially hepatotoxic doses of EBRT. In HCC patients, the severity of cirrhosis has been found to be the predominant risk factor for the development of RILD[139-141]. In addition to RILD, other potential side effects of EBRT include fatigue, nausea, vomiting, and late gastrointestinal bleeding or ulceration if the target is located in close proximity to visceral organs.

Technological advances in the field of radiation oncology such as sophisticated radiation treatment planning and advanced imaging have allowed the ability to deliver high tumoricidal doses of radiation to a partial volume of the liver shaped closely to tumors (conformal EBRT). To date, published randomized data are lacking that have studied the efficacy of EBRT in comparison to alternative treatments or supportive care. However, the collective experience in treating HCC with EBRT is rapidly growing.

Phase Ⅱ prospective and retrospective studies of conformal EBRT have used a range of moderate radiation doses (generally ≥ 45 Gy, 25-66 Gy) with various conventional or hypofractionated schemes (generally 1.8-2.0 Gy per fraction, 1.5-6 Gy) primarily inpatient with well-compensated liver function (Child-Pugh A cirrhosis 77%-100%) and high-risk tumors that were unsuitable for or refractory to other liver-directed therapies[142-145]. Despite these high-risk features, clinical outcomes have been encouraging with 1-year overall survival of 45%-65%, 1-year local control of 69%-81%, and RILD rates of approximately 15%. Caution in delivering EBRT to patients with more compromised liver function was underscored in a study from China that reported RILD in 60% of Child-Pugh B patients and an overall fatality of 85% in any patient that developed RILD[143].

Stereotactic body radiation therapy (SBRT) is a more recent technology that combines stereotaxy (accurate 3D target localization) and multiple finely collimated radiation beams to more precisely deliver ablative doses of radiation (24-54 Gy) over a small number (1-6) of fractions.SBRT is typically delivered to relatively small tumors 1-5 cm in size that are not in close proximity to visceral organs such as the stomach or bowel. The treatment planning and delivery of liver SBRT are complex. Robust assessment of tumor/organ motion and accurate image guidance are essential componentsin light ofthe high radiation doses that are delivered over steep dose gradients[146].

Phase Ⅰ and Ⅱ prospective trials, primarily in Child-Pugh A cirrhotic patients, have demonstrated comparable therapeutic efficacy compared to other liver-directed ablative therapies with 1-year overall survival and local control of 55%-75% and 75%-90%, respectively[147-149]. RILD reported as Child-Pugh deterioration of at least 2 points at 3 mo varies from 13%-29%. One study reported a decrease in RILD of 29% at 3 mo to 6% at 12 mo, suggesting the potential for hepatic recovery after RILD. Multiple modern retrospective studies confirm these prospective data: 1- to 2-year overall survival of 64%-79% and local control of 88%-100%[150-153]. When compared to a matched-pair cohort of patients managed with supportive care only, liver SBRT was found to improve overall survival from 42% to 73% at 2 years. In regards to treating Child-Pugh B patients, recent prospective data also highlight caution in treating Child-Pugh B patients with liver SBRT since Child-Pugh progression was noted in 63% of patients[154].

Charged particle radiation, such as protons and carbon ions, is a form of EBRT that has been employed to treat HCC due to its physical properties that allow the majority of the radiation energy to be deposited over a narrow range of tissue depth (Bragg peak) with little to no exit dose deposited beyond the Bragg peak. This unique dose deposition characteristic may allow higher doses of radiation to be delivered to HCC tumors and lower doses to surrounding normal tissues when compared with photon-based forms of EBRT; a theoretical advantage that is especially appealing in HCC patients where sparing as much remnant liver function as possible is critical.

Protons have been the most commonly studied charged particle in the treatment of HCC with the vast majority of experience from Japan. Multiple phase Ⅱ prospective and retrospective studies using a hypofractionated approach delivering 63-77 GyE over 10-22 fractions have reported effective tumor control rates and low hepatotoxicity of < 10%[155-158]. Local control for small (< 5 cm) and large (5-10 cm) tumors has been excellent with protons, ranging from 81%-88% at 5 years in survivors[156,157]. Local control for high-risk bulky tumors (> 10 cm with portal venous thrombosis in 50%) has also been encouraging (87% at 2 years)[158].

EBRT has been explored in multiple other settings. Limited data with small numbers of patients investigating SBRT as bridging therapy for liver transplant when other bridging therapies were not suitable have shown that SBRT can be delivered safely without risk of intraoperative complications or long-term clinical

compromise and effectively with at least some degree of pathologic response and pathologic complete response in 82%-100% and 20%-27% of tumors, respectively[159-161]. EBRT has been reported to treat HCC with portal venous thrombosis with the goals to restore portal flow for hepatic function maintenance and/or to eliminate arteriovenous shunting to allow successful future delivery of catheter-based therapies. The largest retrospective study to date with 412 patients treated with a combination of TACE and EBRT had a median survival of 11 mo, radiographic response rate of 40% and portal venous thrombus progression-free rate of 86%[162].

Thus, EBRT has the potential to be used in many different settings for the management of HCC: an alternative treatment for tumors that are unsuitable for other liver-directed treatments, salvage therapy for tumors refractory to other therapies, bridging therapy for liver transplant, and combination therapy to complement other treatment modalities. Additional prospective and randomized studies are needed to more clearly define its role in the routine management of HCC. The results of RTOG 1112, an active randomized phase Ⅲ trial studying the role of SBRT (photons or protons) in addition to sorafenib in high-risk HCC patients, are eagerly awaited.

SORAFENIB IN THE TREATMENT OF ADVANCED HCC

The majority of patients diagnosed with HCC present with disease not amenable to surgical or potentially curative intervention[51]. Systemic therapy with sorafenib confers modest prolongation of overall survival and transient disease stability in appropriately selected patients with locally advanced or metastatic disease. Clinical investigations of new systemic agents suggesting efficacy and acceptable tolerance in patient with underlying cirrhosis have shown promise, and will hopefully fill the high unmet clinical need in the coming years.

Historically, cytotoxic chemotherapy including doxorubicin monotherapy was employed in the therapy of advanced HCC over several decades with negligible clinical benefit over supportive care without definitive survival advantage[163,164]. More recently, two pivotal phase Ⅲ clinical trials (SHARP and Asia-Pacific) have established sorafenib as the current standard of care for first line systemic therapy of advanced HCC[165,166].

Sorafenib, an oral multikinase inhibitor affects multiple relevant cellular mechanisms based upon preclinical models including inhibition of neovascularization and cellular proliferation, in addition to induction of apoptosis. Key molecular targets thought to contribute to antitumor efficacy include VEGF, platelet derived growth factor recepter (PDGFR)-B, and RAF kinase inhibition[167]. The SHARP trial was a large randomized double-blind phase Ⅲ trial comparing sorafenib 400 mg twice daily *vs* placebo and best supportive care[165]. This trial enrolled patients with advanced HCC naïve to systemic therapy, the vast majority of whom had Child-Pugh A or better hepatic function and ECOG performance status of 0-2. A statistically significant improvement in both overall survival (10.7 mo *vs* 7.9 mo) and time to disease progression (5.5 mo *vs* 2.8 mo) favored the active therapy arm, despite a low documented response rate of 2%. While the SHARP trial included mainly patients from European or North American sites with HCV and alcohol-related risk factors, significant improvements with sorafenib compared to placebo were also documented in a predominantly Asian, Hepatitis B infected population in the Asia-Pacific trial[166]. This second large phase Ⅲ trial demonstrated a significant improvement in overall survival (6.5 mo *vs* 4.2 mo) favoring sorafenib.

Clinical observations based upon exploratory subset analyses suggest a relative advantage to patients with Hepatitis C related disease, while patients with Hepatitis B may achieve less benefit from sorafenib[167]. Notably, high quality data exists for patients with relatively preserved hepatic function, and the benefit of sorafenib in patients with Child-Pugh B cirrhosis remains unclear. The prospective, non-interventional phase Ⅳ GIDEON trial has documented a similar time to progression in Child-Pugh A *vs* Child-Pugh B populations, but higher rates of serious adverse events and dramatically lower overall survival rates in the Child-Pugh B populations (5.2 mo), bringing into question the relative benefit of sorafenib in the Child-Pugh B subgroup[168,169].

At this time, there is no data to suggest benefit of sorafenib in the adjuvant setting based upon preliminary results of the STORM trial. In this large phase 3 study, 1114 patients with HCC who had undergone surgical resection or RFA with curative intent were randomized to adjuvant sorafenib or placebo, with no differences detected in relapse free survival, time to recurrence or overall survival to date[170]. Additionally, the combination of sorafenib with catheter-based therapy remains of unclear clinical benefit and should only be considered in the context of clinical investigations at this time. Randomized phase 2 data from the SPACE trial assessing the potential impact of adding sorafenib to serial drug-eluting bead chemoembolization in intermediate-stage HCC patients, while meeting the predefined primary endpoint of improved time to progression (HR = 0.8, 95%CI: 0.59-1.08), showed no overall survival benefit and the expected toxicities predicted from combination therapy[171]. At this time, further investigation in phase 3 trials is necessary prior to adopting such a strategy, as the clinical benefit of such combinations remains unclear and benefits in delayed progression associated with early introduction of sorafenib may be outweighed by increased toxicity and impaired quality of life.

Thus, Sorafenib provides a transient period of disease stability despite low overall response rates, translating to a modest survival advantage in patients with advanced HCC (including macroscopic vascular invasion or metastatic disease) and Child-Pugh A or better hepatic function based upon these two trials. Side effects such as fatigue, nausea, diarrhea, anorexia, and weight loss have been documented and may prompt

dose reductions. The common side effect of hand-foot skin reaction can be minimized by introduction of twice daily urea-based skin cream based upon randomized data, and anti-diarrheals and antiemetics play a key role in symptom management[172]. While this agent represents the current standard of care for systemic therapy in HCC, further clinical trials of new agents with enhanced efficacy and improved toxicity profile remains critical.

EXPERIMENTAL AGENTS FOR THE TREATMENT OF ADVANCED HCC

Enhanced understanding of the molecular pathogenesis of HCC and the parallel development of novel targeted therapeutics has led to a dramatic increase in interventional trials targeting advanced HCC over the past several years. Further investigation into the role of optimizing anti-angiogenic therapy, fine-tuning the spectrum of inhibition with various multi-kinase inhibitors, targeting of the hepatocyte growth factor/c-Met pathway, and early investigation into the role of immune checkpoint blockade agents provide some notable examples of current trends in clinical investigation and will be briefly summarized below.

Multiple signaling pathways contribute to angiogenesis in HCC, with subsequent attempts to target such mediators for therapeutic benefit representing a viable approach. VEGF is over-expressed in HCC and optimal inhibition of the ligand and its receptors remains an active area of investigation. Additionally, targeting of fibroblast growth factor (FGF), PDGF and angiopoietin 1/2 contribute to angiogenesis and represent rational targets for therapeutic intervention[173]. To date, trials attempting to enhance the anti-angiogenic effect of sorafenib, either through direct and potent targeting of the VEGF axis alone (*i.e.,* VEGFR2 inhibitor Ramucirumab), through an altered spectrum of inhibitory targets (*e.g.,* Brivanib inhibition of VEGFR/FGFR), or by modulation of the inhibitory profile of sorafenib (sunitinib, linifanib) have failed to demonstrate an advantage over Sorafenib in randomized phase 3 studies through lack of efficacy, increased toxicity, or both. In short, sorafenib has been surprisingly difficult to improve upon with such strategies, although currently lenvatinib, a multi-kinase inhibitor targeting VEGFR1-3, FGFR1, PDGFR a/b, remains under investigation in first line trials compared to sorafenib given promising phase 2 survival data and a reasonable toxicity profile[174].

Preclinical data suggests that Hepatocyte growth factor and its receptor c-Met play a key role in HCC angiogenesis, metastasis and cellular proliferation[175]. Targeted therapy of c-Met is currently under evaluation in several trials including two randomized phase 3 studies in advanced HCC after initial treatment with sorafenib. Previous clinical trial data suggests high tumoral c-Met expression is associated with poor overall prognosis compared to c-Met low HCC[176]. Additionally, data from a randomized, placebo-controlled phase 2 trial including patients with unresectable treatment-refractory HCC treatment with an oral c-Met inhibitor tivantinib was associated with significantly improved survival compared to placebo among the approximately 60% of patients with Met-high tumors (7.2 mo *vs* 3.8 mo, HR = 0.38). Trials of cabozantinib, a combination VEGFR2/c-Met inhibitor have shown promising median overall survival (15.1 mo) and time to progression data in a phase 2 randomized discontinuation study. Taken in combination, data supports continued investigation of c-Met inhibitors in advanced HCC populations and the potential for future biomarker driven selection of systemic therapy[177].

Immune checkpoint inhibition with agents targeting Programmed cell death 1 (PD1), it's ligand programmed death ligand 1 (PD-L1), Cytotoxic T lymphocyte associated antigen 4 (CTLA-4) or other targets have shown dramatic results in multiple malignancies typically deemed refractory to more standard chemotherapy[178]. Immune checkpoint proteins elicit signals (often present in the tumor) limiting anti-tumor immune response, but such inhibitory signals can be negated with a variety of new agents allowing a more robust tumor specific T-cell repertoire. HCC is a rational target for such immune modulation based upon preclinical and observational data[179]. First, multiple case reports of spontaneous regression presumably due to immune response exist[180]. Second, there are observations of improved clinical outcomes after catheter-based therapy for HCC patients with higher titers of tumor-associated antigen specific T-cell subsets[181]. Third, higher expression of tumoral PD-L1 (and resultant inhibition of immune response) in resected HCC has been associated with worse overall survival and more rapid time to progression[182]. These observations and others have led to early investigation of immune checkpoint inhibitors in patients with HCC. Notably, a phase Ⅰ trial of the CTLA-4 inhibitor tremelimumab in patients with hepatitis C and advanced HCC demonstrated an impressive 18% response rate with 45% of patients experiencing disease stability for over 6 mo with acceptable toxicity profiles. Currently, phase Ⅰ trials of the PD1 inhibitor nivolumab are accruing in patients with HCC and Hepatitis B, C or no viral infection. The results of such studies are eagerly anticipated. Future combinations of immune checkpoint inhibitors in combination with local therapy or as adjuvant therapy should be strongly considered if initial trials demonstrate promising response rates and adequate safety profiles.

While cytotoxic chemotherapy alone has not demonstrated significant benefit, the combination of doxorubicin and sorafenib may show synergistic effects and is currently under evaluation in an ongoing randomized phase 3 trial. This trial builds on a completed randomized phase 2 data demonstrating significant benefit of the combination over doxorubicin alone, with improved overall survival (13.7 mo *vs* 6.5 mo) and time to progression (6.4 mo *vs* 2.8 mo)[183]. While initial

data has suggested potential benefit when compared to historical controls of sorafenib monotherapy trials, a randomized comparison against sorafenib is required to establish superiority and tolerance of the combination.

These areas of investigation represent an overview of current trends in the effort to identify active systemic agents for the treatment of advanced HCC. Additional investigations in enzymatic therapy targeting impaired tumoral arginine metabolism, novel antibodies targeting HCC specific antigens, and modification of relevant pathways such as transforming growth factor-β and Wnt/β-catenin represent additional areas in development in this rapidly evolving field of clinical investigation.

Faced with an increasing incidence of HCC, the scope of treatment options has broadened significantly over the last decade to benefit a larger proportion of patients. The number and diversity of diagnostic modalities for HCC have also evolved over the past decade. Beyond the current guidelines outlined in the Barcelona Center for Liver Cancer staging system, a number of therapeutic modalities including radiation (either through radioembolization or external beam radiation), irreversible electroporation, and systemic drugs besides sorafenib are being incorporated into the treatment armamentarium for patients with HCC. High-volume centers such as the Liver Tumor Clinic at the University of Washington provide a multi-disciplinary approach that plays an important role in "designing" an appropriate treatment program based on the patient's tumor, underlying liver disease and overall health. Many clinical trials are currently ongoing to explore new "druggable" targets and treatment combinations. In parallel, basic investigations into the molecular mechanisms of disease are shedding new lights into the pathogenesis of a highly heterogeneous set of tumors collectively classified as HCC. Findings from the "bench" science are expected to yield novel strategies towards disease classification, detection, treatment and prevention.

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REVIEW

"Weighing the risk": Obesity and outcomes following liver transplantation

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Abstract

Obesity is on the rise worldwide. As a result, unprecedented rates of patients are presenting with end stage liver disease in the setting of non-alcoholic fatty liver disease (NAFLD) and are requiring liver transplantation. There are significant concerns that the risk factors associated with obesity and the metabolic syndrome might have a detrimental effect on the long term outcomes following liver transplantation. In general, short term patient and graft outcomes for both obese and morbidly obese patients are comparable with that of non-obese patients, however, several studies report an increase in peri-operative morbidity and increased length of stay. Continued studies documenting the long-term outcomes from liver transplantation are needed to further examine the risk of recurrent disease (NAFLD) and also further define the role risk factors such cardiovascular disease might play long term. Effective weight reduction in the post liver transplant setting may mitigate the risks associated with the metabolic syndrome long-term.

Key words: End stage liver disease; Obesity; Morbid obesity; Non-alcoholic steatohepatitis; Non-alcoholic fatty liver disease; Cirrhosis; Liver transplantation

Core tip: Cirrhosis in the setting of obesity especially from non-alcoholic fatty liver disease is quickly becoming one of the leading indications for liver transplantation. These patients present unique challenges both at the time of transplant and long term secondary to chronic illnesses associated with the metabolic syndrome. Outcomes following liver transplantation and management of these patients will be discussed in light of the current available literature.

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INTRODUCTION

Obesity or the excessive accumulation of body fat contributes to a host of chronic health problems. Obesity is defined by a body mass index greater then 30 whereas severe obesity, morbid obesity and super obesity are defined by a body mass index (BMI) ⩾ 35, 40, and 50, respectively[1]. Obesity rates have continued to sore throughout the world as populations continue to adopt a more "Western" type of lifestyle. A diet of highly processed, refined foods, fat, and red meats has also been linked to increase rates of cardiovascular disease and cancer. Based on recent statistics, it is estimated that greater then 2.1 billion people in the world are either overweight or obese[2]. In the United States, there are approximately 30 million people who are overweight equaling greater than 30% of the population[2].

Obesity is associated with the clinical condition known as the metabolic syndrome that includes hypertension, hyperlipidemia, hyperglycemia, and increased abdominal fat deposition[3]. In addition to obesity, insulin resistance has also been found to be associated with the metabolic syndrome[4]. Individuals with a diagnosis of metabolic syndrome are at increased risk for cardiovascular events, stroke, diabetes, and chronic liver disease.

The natural course of chronic liver disease is progression to cirrhosis and end stage liver disease (ESLD) if the inciting factor(s) is not controlled. ESLD leads to portal hypertension and a host of other complications including gastrointestinal bleeding, encephalopathy, jaundice, ascites, malnutrition, and hepatocellular cancer (HCC). Although there are multiple causes of chronic liver disease and cirrhosis (*e.g.*, viral hepatitis, autoimmune hepatitis, cholestatic liver diseases, excessive alcohol consumption), the most common cause of chronic liver disease in the United States is now non-alcoholic fatty liver disease (NAFLD) or its more aggressive form, non-alcoholic steatohepatitis (NASH)[5].

Obesity and its associated complications is thought to have a significant impact on post-operative outcomes and survival after surgical procedures. The allocation of health resources and the expense associated with caring for obese patients continues to be controversial especially in light of the current changing health care landscape. Nowhere is this more true than in the field of liver transplantation in which there is not only an obligation to provide cost effective care but also the responsibility of allocating a scarce resource.

NAFLD, NASH, and obesity

NAFLD is thought to be the hepatic manifestation of the metabolic syndrome. NAFLD encompasses a spectrum of clinicopathologic disease ranging from hepatic steatosis (in the absence of significant alcohol consumption) to the more aggressive form NASH in which fatty deposition and necroinflammation are present. Pathologically, NASH is characterized by macrovesicular steatosis, ballooning degeneration with or without Mallory bodies, and lobular or portal inflammation, with or without fibrosis[6]. The majority of patients with NAFLD have a benign course and many have stable fatty liver disease. NASH is thought to result from a two-hit insult in which accumulation of fat is the first step but an additional stressor is necessary in order to lead to progressive liver damage and NASH[7]. Interestingly, there also appears to be a genetic predisposition to developing NAFLD and NASH[8]. In addition, there are racial differences observed in the prevalence of NAFLD in the United States with the highest prevalence found in Hispanics, followed by Caucasians, and then African-Americans[9-11].

It is estimated that greater than 25% of patients with NASH will develop progressive fibrosis over time with approximately 10%-20% of patients eventually developing advanced fibrosis or cirrhosis[12]. Clinical risk factors for progression of fibrosis include insulin resistance and hypertension[13]. NASH likely accounts for a large portion of cases that were previously labeled as cryptogenic cirrhosis especially those cases of cirrhosis that occur in the setting of obesity, diabetes, and cardiovascular disease. Further support for the link between NASH and cryptogenic cirrhosis comes from the fact that patients transplanted for cryptogenic cirrhosis have a high prevalence of NAFLD and NASH in their post transplant grafts[14].

The incidence of NAFLD in Western countries is estimated to be between 20%-30%[15]. Currently in the United States, NAFLD and NASH are the leading causes of chronic liver disease and NAFLD is estimated to effect approximately 30% of the general United States population and up to 90% of people with morbid obesity. NASH is thought to affect about 5%-13% of the general population, and studies have shown the presence of NASH in 31% of patients with a clinical diagnosis of NAFLD on ultrasound[9].

NASH in pediatric patients

The obesity epidemic has not spared the pediatric population in the world. Typically thought of as a disease of adulthood, obesity rates and the incidence of NAFLD and NASH have skyrocketed in the pediatric population. Like adults, NAFLD now is the most common cause of chronic liver disease in children and adolescents[16]. An autopsy study found that 9.6% of the American population aged 2-19 years old have NAFLD, and the percentage increased to 38% among those who were obese[17]. NAFLD appears to be more prevalent in the older adolescents as compared to younger children and is also more common in boys (ratio of 2:1)[18]. NAFLD is more prevalent in the Mexican communities and also in children from Asian-Indian and Asian-American descent[19,20]. Rates in Asian patients are thought to potentially be due to increased rates of insulin resistance

and visceral adiposity[19]. The long-term outcomes of pediatric NAFLD are not well known and are actively being investigated. A recent study, however, suggests that biopsies from adolescents with NAFLD have significantly higher incidence of NASH, hepatocyte injury scores and fibrosis when compared to a similar group of adults. The authors concluded that adolescents with severe obesity have more advanced liver damage and more severe systemic inflammation than adults suggesting differences in NAFLD etiologies and more aggressive disease progression in the young obese population[21].

The data on NAFLD/NASH progression and the need for liver transplantation are scant. However, in one study, 66 children with NAFLD were followed for up to 20 years and 2 of these children underwent liver transplantation for decompensated cirrhosis. In both of these children, NAFLD recurred in the allograft with one case progressing to cirrhosis requiring retransplantation[22]. Further studies are needed to identify those children that are at higher risk for progression of NAFLD to NASH and ultimately might require liver transplantation.

Obesity and HCC

One of the major indications for liver transplantation is HCC, especially when the tumor is multifocal or when it occurs in the setting of chronic liver disease. Obesity has been identified in several studies as a clear risk factor for the development of HCC[23-25]. In the case of NAFLD, it is estimated that HCC occurs in up to 27% of patients with cirrhosis, but even patients without NASH are at risk for developing HCC[26]. The etiology of HCC in NAFLD and NASH is thought to be related to chronic inflammation and repeated injury to hepatocytes from the accumulation of fat in the liver. Interestingly, recurrence-free survival in patients with HCC in the setting of NASH appear to be significantly better than in the setting of hepatitis C virus (HCV) for both resection and liver transplantation[27,28].

OUTCOMES FOLLOWING LIVER TRANSPLANTATION IN OBESE INDIVIDUALS

Early reports of liver transplantation in obese recipients demonstrated mixed outcomes perhaps because of small sample sizes. Studies have also included heterogeneous groups of recipients; some focus simply on obese patients (BMI $\geqslant$ 30) whereas other studies differentiate between obese patients and morbidly obese patients (BMI $\geqslant$ 40). Initial single center reports showed equivalent short term survival rates for obese and severely obese patients with some centers also documenting higher complication rates (especially wound infections) and higher health care costs for transplantation[29-32]. More recently, there have been several single center and multi-center studies that have focused on preoperative and long outcomes following liver transplantation.

Outcomes of obese patients: Peri-operative morbidity and length of stay

Liver transplant recipients have a significant survival advantage as compared to patients that continue on the waiting list regardless of their BMI at the time of transplant[33,34]. Greater peri-operative morbidity and increased post-operative length of stay appears to be a fairly consistent but not absolute finding in the obese and/or morbidly obese patients in the studies examined (Table 1). In the studies that document a higher morbidity in obese patients, wound related and infectious complications appear to predominate[35-38]. In one study, obese patients surprisingly did not require prolonged ventilatory support as compared to non-obese patients[39].

The differences seen in peri-operative morbidity amongst the different studies can potentially be explained by the heterogeneity amongst the obese and morbidly obese patients in that co-morbid conditions were not taken into consideration. Several studies attempted to take into account these co-morbid conditions. One group examined obesity along with the presence of coronary artery disease, and hypertension and calculated patients' risk of post-operative events. The presence of obesity and diabetes appeared to be the strongest predictors of post-operative events[35]. The presence of cardiovascular risk factors however did not alter the peri-operative risk[35]. Similarly, Nair *et al*[40] examined the combination of several pre-operative risk factors that included obesity and diabetes to create a risk score and tested its ability to predict post-operative outcomes. Compared to the prior study, they found no difference in pre-operative morbidity and length of stay between the low risk and high-risk groups. Neither study found that these conditions affected short-term survival.

Cardiovascular events are a significant cause of morbidity in the post liver transplant patients, which maybe related to the higher prevalence of risk factors associated with the metabolic syndrome such as hypertension and hyperlipidemia in these patients[41]. A similar small study also found an increased risk of cardiovascular events in post liver transplant patients although this did not seem to correlate with obesity as the normal weight cohort had a similar rate of cardiovascular events[42]. A more recent, larger study using the OPTN database attempted to identify predictors of early cardiovascular events[43]. A total of 1576 deaths in the first 30 d post transplant were identified out of 54697 liver transplant recipients of which 42.1% were secondary to cardiovascular events. Surprising, obesity and complications of the metabolic syndrome were not found to be independent predictors of early cardiovascular mortality. Several other recipient factors were found to be significant predictors including pre-operative hospitalization, intensive care unit and ventilator status, and the presence of portal vein thrombosis. Interestingly, Ayala *et al*[44] also found that obesity was a risk factor for pre-transplant portal vein thrombosis.

Table 1 Outcomes following liver transplantation in obese patients (2000-present)

Ref.	Patients (*n*)	Classification (BMI)	LOS	Perioperative complication rate	Graft survival	Patient survival
Nair *et al*[31]	121	NW *vs* OB (27.8-31.1 M and 27.3-32.3 F) MO (> 31.1 M and > 32.3 F)	In MO	In MO	NA	NoD
Nair *et al*[45] (UNOS data)	18172	NW *vs* SO (35.1-40) MO (> 40)	NA	NA	NoD (1 and 2 yr)	In SO (5 yr) In MO (1, 2 and 5 yr)
Dare *et al*[35]	202	NO (< 30) *vs* OB (≥ 30)	In OB	In OB	NA	NoD
Tanaka *et al*[38]	507	cBMI (≤ 40 *vs* 40) mBMI (≤ 40 *vs* 40)	In MO ND	NA	In MO NoD	In MO NoD
Hakeem *et al*[37]	1325	NW *vs* OW (25-29.9), OB (30-34.9) MO (≥ 35)	In OW and OB	In OW and OB	NoD	NoD
Dick *et al*[51] (UNOS data)	73538	NW *vs* MO (≥ 40)	In MO	In MO	NA	In MO
Perez-Protto *et al*[50]	230	NW *vs* OB (≥ 38)	NoD	NoD	NoD	NoD
Fujikawa *et al*[85]	700	NW *vs* OW (25-29.9) OB (≥ 30)	NoD	NoD	NoD	NoD
Hillingsø *et al*[52]	365	NW *vs* OB (> 30)	NoD	NoD	NA	In OB
Conzen *et al*[53]	785	NW *vs* MO (≥ 40)	NoD	NoD	NoD (at 3 yr) In MO (at 5 yr)	NoD (at 3 yr) In MO (at 5 yr)
Werneck *et al*[39]	136	NW *vs* OW (25-29.9) OB (≥ 30)	NoD	NoD	NoD	NoD
Nair *et al*[40]	193	NW *vs* MO (≥ 40)	In MO	NA	NA	NoD
Singhal *et al*[49] (SRTR)	12445	NW *vs* MO (≥ 40)	In MO	NA	NoD	NoD
Schaeffer *et al*[86]	167	NW *vs* OB (> 35)	NA	In OB	NoD (at 1 yr)	NoD (at 1 yr)
Orci *et al*[48] (SRTR)	38194 (4138 > 5 yr)	NW *vs* OB (> 35)	NA	NA	NA	NoD (> 5 yr)

NA: Not available; NoD: No difference; NO: Non-obese; OW: Overweight; OB: Obese; SO: Severely obese; MO: Morbidly obese; BMI: Body mass index; NW: Normal weight; LOS: Length of stay; cBMI: Calculated BMI; mBMI: Modified BMI.

Long-term outcomes

The long-term outcomes of patients with obesity and morbid obesity have yet to be fully determined. One would assume that persistence of obesity and the metabolic syndrome post transplant would clearly put these patients at higher risk for developing serious cardiovascular disease including myocardial infarction and stroke. In 2002, Nair *et al*[45] published a review of the UNOS database from 1988 to 1996 comparing outcomes following liver transplant for patients that were obese, severely obese, and morbidly obese. A total of 18172 patients were examined and the authors found an increased risk of primary non-function and an increased risk of mortality at 30 d, 1 year, 2 years, and 5 years in the morbidly obese group. The severely obese group also had an increased risk of mortality at 5 years. All obese patients (BMI > 30) had an increased risk of death from cardiovascular events[45]. This led to the recommendation by the American Association for the Study of Liver Disease that morbid obesity was a contraindication to liver transplant[46]. Similarly, in 2003, Rustgi *et al*[47] published their analysis of the UNOS database from 1992 to 2000 examining a total of 26920 patients. In this study, patients with BMI ≥ 40 were found to be at increased risk of post-transplant death.

Conversely, a more recent publication examining the SRTR database from 2004 to 2011 identified 38194 recipients of which 8196 were considered obese. Unlike the review by Nair *et al*[45], they found no risk of increase mortality across the different categories of obese patients as compared to the control group. In fact, the authors found a protective affect of overweight male recipients but not female recipients with BMIs ranging between 25 and 35[48]. Another center also recently examined a very similar cohort of patients (SRTR database from 2007-2011) and also confirmed no difference in short-term outcomes. They, however, noted increased resource utilization by patient with BMIs ≥ 40 with more patients disabled in the pre-operative setting and longer post operative hospital stays[49].

Several single center studies have also documented long term outcomes. One of the largest series examined 1325 patients from Leeds, United Kingdom and the authors report no significant difference in graft and patient survival up to 10 years post transplant[37]. Another large single center study found an increased rate of the metabolic syndrome in their post transplant patients with BMIs ≥ 38, however, this did not correlate with poor 3 years outcomes[50]. A smaller study from Ireland also found no difference in long-term survival between their obese patients and non-obese patients [36]. Conversely, a study that examined outcomes over an extended period of time, dividing patients into different eras, found a consistent improvement in outcomes over time. However, in all eras, survival of the morbidly obese patients was worse, and morbid obesity was an independent predictor of death[51]. Interestingly, the risk of morbid obesity appeared to be exacerbated in the MELD era in this study with the poorest long-term survival seen in morbidly obese patients with MELD 22. A Danish group also had similar poor outcomes for their obese group[52]. Another single center study which examined long term outcomes (≥ 5 years) noticed a significant decline in both graft and patient survival at 5 years despite similar outcomes at 3 years[53].

Studies that do demonstrate poor long-term out-

comes associated with obesity have been criticized by the fact that they do not take into account malnutrition, low albumin levels and ascites. In one study, conventional BMI appears to be able to be mitigated by conversion to modified BMI that takes into account low albumin levels and fluid accumulation. When converted to a modified BMI (calculated by multiplying the serum albumin by the BMI), there was no difference in long-term survival of the different groups[38]. Similarly, Leonard *et al*[54] found that when correcting BMI for ascites, up to 20% of patients moved to a lower BMI category. Furthermore, the corrected BMI was not a predictor of poor long-term outcomes.

The inconsistency in results reported from different centers would suggest that patient selection plays a critical role in the outcomes of obese patients after transplant. The more recent trend in better outcomes may reflect better patient selection and improved care in the more recent era. However, more long-term data looking at 5 years and beyond are needed in order to adequately characterize the long-term risk to obese and morbidly obese patients.

Recurrent disease in obese patients following liver transplantation

The risk of recurrent NAFLD in post transplant patients has been documented and ranges between 25%-60%[55-58]. In one study, 39% of recipients transplanted for NAFLD had either recurrent NAFLD or NASH with the strongest independent predictors of recurrence being high pre transplant and post transplant BMIs. The presence of recurrent disease, however, did not appear to affect overall survival at least in the short term[56]. Similarly, patients transplanted for cryptogenic cirrhosis (of which several of the patients were believed to have NASH) have also been shown to develop NAFLD or NASH in the post transplant setting[59]. A more recent study examined the prevalence of post liver transplant NAFLD in patients transplanted for non-NAFLD related liver disease and found steatosis in 40% of patients[60]. BMI pre- and post-transplant appeared to correlate with the risk of developing post transplant steatohepatitis. Commonly used immunosuppressive medications such as steroids and calcineurin inhibitors could be potential factors contributing to insulin resistance/hyperglycemia, hyperlipidemia, and hypertension. In fact, exposure to a high total dosage of glucocorticoids has been associated with the development of NASH[61].

The association between chronic HCV infection and post transplant diabetes is well known. HCV appears to be partially responsible for inducing insulin resistance and diabetes mellitus. Unfortunately, the presence of diabetes mellitus portends a bad outcome with patients suffering from an accelerated progression to fibrosis leading to poor graft and patient survival[62].

Obesity also appears to affect recurrence of HCC. In a study by Mathur *et al*[63], the authors demonstrate a doubling in recurrence rates of HCC in both overweight and obese patients as compared to a lean group of patients following liver transplant. Similarly, patients with a BMI > 25 had more accelerated time to recurrence.

SPECIAL CIRCUMSTANCES

Bariatric surgery prior to liver transplantation: Lessons learned from the jejunal-ileal bypass surgery

There is no question that a prior history of upper abdominal surgery can increase the risk of peri-operative complications at the time of liver transplant. Abdominal scarring and adhesions can increase the complexity of the initial hepatectomy. In addition, vascularization of these adhesions from portal hypertension can result in greater blood loss. Many morbidly obese patients have attempted weight loss surgery prior to transplantation which puts the remnant stomach at risk for devascularization and luminal perforations.

The jejunoileal bypass (JIB) was a bariatric procedure that was performed with high frequency in the 1960 and 1970s. This weight loss procedure consisted of dividing and anastomosing the first 35 centimeters of proximal jejunum to the terminal 10 centimeters of ileum in an end-to-side or end-to-end fashion[64]. Although this procedure was effective in causing malabsorption and weight loss, it also carried the complication of chronic liver disease and in some cases acute liver failure[65].

There have several reports that have documented the feasibility of performing liver transplants in patients who had previously undergone a JIB. Although the reports were small series, they documented that transplant was feasible with reasonable patient outcomes. In all cases, reversal of the bypass appears to be critical for the prevention of recurrent disease in most patients[66,67]. More recently, there have been several reports of patients requiring transplant after a biliopancreatic diversion (Scopinaro procedure or duodenal switch) from massive steatosis and sub-fulminant hepatic failure[68,69].

A recent survey of transplant centers in Belgium identified patients that had undergone liver transplant after bariatric surgery. They identified 10 patients listed for liver transplantation with a mean time to wait listing post bariatric surgery of 5 years. The majority of the patients (9 of 10) had undergone biliopancreatic diversion. Of the 10 patients, 7 were transplanted, 2 died waiting for transplant, and one was still waiting at the time of publication. Of the 7 patients transplanted, 4 patients were still alive. One of the 4 patients required retransplantation at 10 mo due to rapid recurrence of liver disease. Although, liver transplantation can salvage patients with post bariatric surgery liver failure, outcomes appear to be poor and bariatric patients should be monitored closely for liver dysfunction following surgery[70].

Bariatric surgery in conjunction with or after liver transplantation

An attempt at bariatric surgery is appropriate for patients with early stage liver disease[71], but is never indicated in patients with advanced stage liver disease or cirrhosis. For many of these patients, continued long-

term obesity post transplant will undoubtedly increase patients' risk for long term complications associated with the metabolic syndrome. The risk obesity poses to the recurrence of NASH and HCV are also now coming to light. For many post-transplant patients, diet and exercise is rarely enough to incur significant, sustainable weight loss. Bariatric surgery has taken on many different forms (*e.g.*, gastric bypass, sleeve gastrectomy, gastric band) all of which have varying rates of technical complexity, associated complications, and effectiveness in terms of weight loss.

Several groups have documented the safety of performing bariatric surgery on post liver transplant patients either in small studies or case reports. An initial report by Duchini *et al*[72] documented that roux-en-Y gastric bypass (RYGB) could be safely performed in post liver transplant patients. This study was further supported by larger studies by Al-Nowaylati *et al*[73] and Tichansky *et al*[74]. Although RYGB was effective in inducing weight loss, this did not come without risk. Complications post bariatric surgery included dumping, wound infections, and in one severe case, multi-system organ failure and death. One patient required reversal due to intractable malnutrition and gastrojejunal ulcers. Other groups have also shown that a sleeve gastrectomy can be performed safely and is effective in inducing weight loss in the post liver transplant patient[75,76].

Due to the increased technical complexity secondary to adhesions and complications related to long-term immunosuppression, some groups have attempted bariatric surgery at the time of transplant. In an initial report, Campsen *et al*[77] reported safely performing a gastric band in patients immediately after the new liver was transplanted. In a similar approach, Heimbach *et al*[78] from the Mayo Clinic reported their initial experience with performing the gastric sleeve at the time of liver transplant. This was chosen over the gastric band due to increased efficacy in inducing weight loss and the fact that there was no need for a foreign body in an immunosuppressed patient. In their initial report of 7 patients that underwent a combined liver transplant-sleeve gastrectomy, all patient attained weight loss and none developed post liver transplant diabetes or hepatic steatosis. However, one patient did have excessive weight loss and one patient leaked from the gastric staple line. There were no graft failures or deaths in the combined groups.

Living donor liver transplantation

Adult-to-adult living donor liver transplantation (LDLT) has been shown to have outcomes equivalent to deceased donor liver transplantation especially in regions where organ donation is scarce. Death on the waiting list which can be as high as 20% at some United States centers. It is well documented that there is a significant survival advantage to patients transplanted with living donors as compared to those patients that wait on the deceased donor list when compared from time of listing by preventing[79]. In other parts of the world where deceased donation is non-existent, LDLT is the only option for patients with ESLD. Appropriate size matching of the liver graft from the living donor with the recipient is essential for success with most programs using a cutoff graft weight to recipient weight ratio (GRWR) of 0.8. Successful LDLT has been performed with lower GRWR[80] and there is a resurgence of left lobe grafts in the Western world[81].

Appropriate matching of donors with obese recipients can be especially challenging in the setting of LDLT especially when using the common cutoff of 0.8 for the GRWR. Whether this ratio is appropriate in the setting of obesity has yet to be determined. There are no studies that examine the morbidly obese population, and studies examining LDLT in the setting of obesity are scarce. The largest study by Gunay *et al*[82] examined 380 patients who underwent LDLT of which 74 were considered obese (BMI $\geqslant$ 30). No patients were morbidly obese (BMI > 40). Although the obese patients had a harder time finding suitable living donors, the complication rate, graft survival, and patient survival were all similar when comparing the obese recipients to either the overweight or normal weight recipients[82]. A smaller study of 7 patients with NASH of which 6 of the patients were obese also demonstrated that LDLT was feasible, but again these patients appeared to have a more difficult time identifying suitable donors[83]. Further studies are needed to address long-term outcomes of LDLT and also to further investigate the applicability of a GRWR of $\leqslant$ 0.8 in the setting of morbid obesity.

OUR EXPERIENCE WITH MORBID OBESITY AND LIVER TRANSPLANTATION AT OCHSNER MEDICAL CENTER

Over the last few years, Ochsner medical center has grown to become one of the largest liver transplant programs in the United States performing 196 liver transplants in 2014. Due to its geographic location in the South Eastern corridor of the United States, the program has a vast experience with liver transplantation of the morbidly obese patient. In our experience, it is important to make sure that the morbidly obese patients are properly cleared from a cardiopulmonary perspective as many of them can have occult coronary disease and/or pulmonary hypertension. From a technical perspective, line placement and exposure during transplant can be challenging and we have moved to using a Thompson retractor with special bariatric blades to aid in exposure.

A chart review of primary liver or combined liver-kidney transplants was performed between September 2005 and December 2008 of which 255 adult transplants were identified. A comparison of morbidly obese patients (n = 34) *vs* a control group (n = 221) of non-morbidly obese patients was performed and several characteristics including 30 d and 1 year graft and patient survival, length of stay, and 30 d re-operation rate were recorded. Based on our data, morbidly obese patients had longer

median length of stays (19 d *vs* 13 d), but 30-d re-operation rates were not higher in the morbidly obese group. Thirty day and 1-year graft and patient survival were equivalent[84].

CONCLUSION

There are multiple indications for liver transplantation in the obese patient, but NAFLD is the most common. Obese patients appear to be at higher risk for peri-operative complications and length of stay post-transplant is longer which potentially can increase the global health care cost to managing these patients. However, this does not appear to impact both short and long term outcomes following transplant. The impact of obesity and the metabolic syndrome on long-term outcomes remains to be determined but these patients are at risk for recurrent steatohepatitis. Weight reduction post transplant is likely to be effective in avoiding complications of the metabolic syndrome including post transplant diabetes and steatosis. Weight loss surgery appears to be advantageous at the time of transplant since it avoids the need for an additional surgery and also avoids the potential for increased complications due to abdominal scarring and long-term immunosuppression. Appropriate patient selection is critical for minimizing complications and obtaining optimal short and long-term outcomes.

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REVIEW

Intravenous immunoglobulins in liver transplant patients: Perspectives of clinical immune modulation

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Abstract

Shortage of appropriate donor grafts is the foremost current problem in organ transplantation. As a logical consequence, waiting times have extended and pretransplant mortality rates were significantly increasing. The implementation of a priority-based liver allocation system using the model of end-stage liver disease (MELD) score helped to reduce waiting list mortality in liver transplantation (LT). However, due to an escalating organ scarcity, pre-LT MELD scores have significantly increased and liver recipients became more complex in recent years. This has finally led to posttransplant decreasing survival rates, attributed mainly to elevated rates of infectious and immunologic complications. To meet this challenging development, an increasing number of extended criteria donor grafts are currently accepted, which may, however, aggravate the patients' infectious and immunologic risk profiles. The administration of intravenous immunoglobulins (IVIg) is an established treatment in patients with immune deficiencies and other antibody-mediated diseases. In addition, IVIg was shown to be useful in treatment of several disorders caused by deterioration of the cellular immune system. It proved to be effective in preventing hyperacute rejection in highly sensitized kidney and heart transplants. In the liver transplant setting, the administration of specific Ig against hepatitis B virus is current standard in post-LT antiviral prophylaxis. The mechanisms of action of IVIg are complex and not fully understood. However, there is increasing experimental and clinical evidence that IVIg has an immuno-balancing impact by a combination of immuno-supporting and immuno-suppressive properties. It may be suggested that, especially in the context of a worsening organ shortage with all resulting clinical implications, liver transplant patients should benefit from immuno-regulatory capabilities of IVIg. In this review, perspectives of immune modulation by IVIg and impact on outcome in liver transplant patients are described.

Key words: Intravenous immunoglobulins; Immune modulation; Hyperimmunoglobulin; Model of end-stage liver disease; Liver transplantation

Core tip: In times of an escalating organ scarcity, decreasing posttransplant survival rates following liver transplantation have been reported. Predominantly infectious and immunologic complications were identified to account for this recent outcome deterioration. Therefore, balancing the recipients' immune system is currently discussed as useful approach to improve prognosis. Intravenous immunoglobulins (IVIg) are thought to provide favorable immuno-regulatory capabilities. This paper summarizes the current available clinical data that indicate beneficial immuno-modulatory properties of IVIg in liver transplant patients.

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INTRODUCTION

Liver transplantation (LT) has evolved to become a standard procedure in the treatment of end-stage liver disease[1,2]. Due to refined surgical techniques, advancements in intensive care treatment and progress in immunosuppressive medication, post-LT outcome improved dramatically over the past decades[3]. As a result, donors' and recipients' selection criteria were considerably expanded and numbers of LTs performed were significantly increasing in recent years. Due to a dramatic donor organ shortage, growing waiting lists, prolonged waiting times and increasing pre-LT mortality rates have been reported[4-6]. To respond to this challenging situation, the model of end-stage liver disease (MELD) score was implemented to give priority to the most urgent patients on the waiting lists. The "sickest first" approach based on serum creatinine, bilirubin, and the international normalized ratio contributed to reduction of waiting list mortality[7-13]. However, the problems were rather shifted from the pre- to the posttransplant period. It was a consequence of the escalating organ shortage that final pre-LT MELD scores were significantly increasing in recent years[11-14]. Therefore, liver transplant patients became more complex with considerably higher perioperative risk profiles. Rates of early posttransplant immunologic and infectious complications have markedly increased and survival rates were, thus, significantly deteriorating in recent years[10-14]. There is evidence that the immune systems of high-MELD patients are per se compromised, which in turn, may lead to an increased risk of septical disorders. Almost 85% of patients become afflicted with early infections, which is nowadays the most common cause of death soon following LT[10-14]. To realize LT at an earlier stage of disease progression, an increasing number of so-called extended criteria donor organs (ECD; based on donor age, liver steatosis, allograft infections, living-related or non-heart beating donors) are nowadays accepted[15,16]. The use of such marginal grafts may, however, aggravate the risk of allograft dysfunction, immunologic imbalance and infectious complications[15,16]. Therefore, balancing the liver recipients' immune system has been recognized as key approach in the context of organ scarcity and resulting clinical implications. Tailoring the immunosuppressive therapy to the patients' individual need is an established strategy for an early immune regulation[17]. However, balancing between reduction of infectious risks and increased susceptibility for graft rejection may be difficult. Indeed, there are no clinical parameters that reliably define the lowest possible immunosuppressants' dose for avoiding immunologic attacks to the allograft[18]. Need of anti-rejection treatment may, in turn, increase the risk of septical complications[19]. Therefore, a combination of immuno-stimulating and immuno-suppressive properties, as were recently suggested for intravenous immunoglobulins (IVIg), could be another attractive immuno-balancing approach[20-22].

Treatment with IVIg was introduced in the 1950's, primarily for substitution of antibodies in patients with immune deficiencies[20-22]. Since the evidence that IVIg may ameliorate immune thrombocytopenic purpura in 1981, it has been used for the treatment of a wide range of autoimmune and systemic inflammatory disorders. In addition to these mainly antibody-mediated diseases, IVIg proved to be effective in several disorders caused by deterioration of the cellular immune system, like multiple sclerosis, Kawasaki disease and graft *vs* host disease[20-25]. Subsequently, IVIg was increasingly used in the transplant setting. It was shown to be effective in prophylaxis and treatment of severe allograft rejection, particularly in highly sensitized kidney and heart recipients. In addition, IVIg proved to be beneficial in the treatment of posttransplant hypogammaglobulinemia[26-28]. In the 1990's, the use of specific immunoglobulins (Ig) against hepatitis B virus (HBV) was established as standard for prophylaxis against HBV recurrence in liver transplant patients[29].

The exact modes of action of IVIg are complex and not yet fully understood. However, there is increasing experimental and clinical evidence that, beyond clearing pathogenic autoantibodies, IVIg may establish long lasting modulations of the cellular immune system[21,22]. The nature of these immuno-regulatory capabilities suggest that, particularly in these times of higher immunologic and septical risks, liver transplant patients might benefit from early post-LT treatment with IVIg[30,31].

The aim of this review was to report on current available data indicating prognostically favourable immuno-modulatory properties of IVIg and, thereby, improved outcome following LT. For this purpose, an extensive review of the English literature using the PubMed database was performed by selecting papers according to the following key terms: "liver transplantation", "immunoglobulin", "hyperimmunoglobulin", and "immune

modulation".

MECHANISMS OF IMMUNE MODULATION BY IVIG

Therapeutically administered Ig consist of a polyspecific IgG preparation with small amounts of IgA and IgM. It is obtained from plasma pools of either thousands of healthy blood donors or donors with specifically high antibody titers directed against several viruses[20-22]. Treatment with IVIg was shown to be safe. Only mild generalized symptoms like headache, fever and nausea have been described in a small number of patients, but serious adverse effects are mostly uncommon. The half-life of IVIg is about three weeks. The clinical effects of IVIg were, however, proven beyond this period. Therefore, immuno-regulatory capabilities by IVIg were suggested to be based not only on antibody-mediated mechanisms but rather on interactions with the cellular immune system[20-22]. The modes of action of IVIg are very complex and still elusive[30]. They are triggered *via* selective and distinct molecular mechanisms of biological processes that are implicated in innate or acquired immune responses[20-22,30]. There are some excellent reviews on the specific effects of IVIg on the immune system[21,22,30,31]. Thus, only some of the most important immuno-regulatory properties of IVIg are mentioned below.

Fab-mediated modes of action

Neutralization of auto-antibodies by anti-idiotype antibodies present in IVIg was one of the first explanations for the anti-inflammatory impact of IVIg. Apart from well-known microbial antigen-specific binding effects, IVIg is supposed to convert a pro-inflammatory trigger into an anti-inflammatory condition by neutralization of endogenous inflammatory chemokines and cytokines and apoptosis-inducing molecules *via* naturally occurring auto-reactive antibodies[32-34].

Targeting of Fc receptors

Fc gamma receptors (FcγRs) are the main receptors for IgG and, thus, very likely to be involved in clinically relevant immuno-regulatory actions of IVIg. They are found on almost all immune cells (B- and T-cells, natural kille cells, dendritic cells, macrophages, monocytes neutrophils, eosinophils, and platelets). They mediate a wide range of biological immune response, like phagocytosis of IgG-opsonized microorganisms or immune complexes, antibody-dependent cellular cytotoxicity, activation of the NADPH oxidase, and the release of cytokines[21,22,33]. Based on their affinity for monomeric IgG, they can be divided in high-affinity FcγRI and the low-affinity FcγRII and FcγRIIII. Biological pathways may be mediated by activating (FcγRI and FcγRIII) or inhibiting (FγRII) mechanisms[34-36]. Blockade of activating FcγRs by high doses of IVIg and, thereby, saturation of FcγRs is discussed as one possible way of immune modulation. Up-regulation of the inhibitory FcγRII as a result of sialylated IgG-Fc is another prevailing theory for immunologic impact of IVIg[36]. Furthermore, saturation of the neonatal FcR (FcRn) may increase the clearance of pathogenic antibodies[37]. FcRn is expressed by human endothelial cells to recycle IgG and, thus, extends its half-life. Saturation of these receptors with high-doses of IVIg is supposed to shorten the half-life of all circulating IgG including harmful auto-antibodies[34,37].

Inhibition of the complement cascade

IVIg was shown to contain antibodies against several components of the classical complement pathways, like C1, C3a, C3b and C4[38]. Apart from that, the Fc portion of IgG was shown to inhibit C5 convertase, an enzyme that is required for subsequent formation of the membrane attack complex[21,22,27].

Effects on cytokines

Modulating the production of cytokines and cytokine antagonists is supposed to be another important immuno-modulatory mechanism of IVIg. This capability is not only triggered by affecting monocytic cytokine production, but also *via* increase of T 1 helper (Th1) and Th2 cytokine gene expression and production[21,39]. IVIg was shown to reduce the level of several cytokines, like interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and nuclear factor κB. Furthermore, it may selectively trigger the production of IL-1-receptor antagonist, the natural antagonist of IL-1[21,33,40]. The anti-inflammatory cytokine IL-11 was, by contrast, shown to be up-regulated by IVIg[41]. Recently, it has been demonstrated that plasma levels of IL-33, IL-4 and IL-13 are increased by IVIg. This molecular mechanism may, in turn, lead to inhibition of inflammatory processes *via* Th2-cytokine-mediated down-regulation of FcγRIIa[42].

Interaction with dendritic cells

Dendritic cells (DC) are a heterogeneous group of antigen-presenting cells that are involved in the pathogenesis of several immune-mediated diseases and allograft rejection[22,43]. IVIg was shown to inhibit differentiation and maturation of human DCs. It may prevent up-regulation of the co-stimulatory molecules CD80 and CD86 that play a crucial role in the interaction between DCs and T-cells[22,43,44]. It is able to minimize the capability of mature DCs to secrete the pro-inflammatory cytokine IL-12, while simultaneously increasing the production of the anti-inflammatory cytokine IL-10[45]. Apart from that, IVIg suppresses DC-related activation and proliferation of auto- and allo-reactive T-cells. Thus, the immunosuppressive properties of IVIg are suggested to be mainly triggered by suppression of DC-specific properties[45,46].

Effects on B-cells

It has been shown that B lymphocytes, unique cells with an Ig as part of the B-cell receptor (BCR), may interact

with IVIg in different ways[47]. Antigen binding to BCR leads to modulation of gene expression, finally resulting in activation, anergy or apoptosis of B-cells. Several co-receptors on the B-cell surface are able to either positively or negatively affect BCR signaling. It has been demonstrated that IVIg may interact with almost all of these co-receptors on the B-cell surface[30,47]. This may lead to other highly relevant B-cell mediated mechanisms of IVIg, including inhibition of B-cell differentiation, inhibition of IL-6 and TNF-α production, induction of B-cell apoptosis, and down-regulation of specific auto-reactive B cells. In addition, IVIg is able to induce secretion of *de-novo* IgG, which may be beneficial in controlling reactivities of pathogenic auto-antibodies[30,47,48].

Effects on T-cells

The capability of IVIg to inhibit human T cell proliferation and cytokine production *in vitro* was shown to be comparable to that of calcineurin inhibitors[46,49]. It is supposed that this inhibitory effect of IVIg on T cells is at least partly caused by suppression of antigen-presenting cells, but also mediated by direct interactions[49,50]. IVIg was shown to suppress proliferation and cytokine production of T-cells by inhibition of IL-2 and interferon-γ production[22,49,50]. In addition, IVIg was demonstrated to contain antibodies against CD4 cells, soluble human leukocyte antigen (HLA) class Ⅰ and Ⅱ molecules, chemokines-receptor CCR-5 and T-cell receptor β chain[21,22,51-53]. It has recently been suggested that a major mechanism of IVIg to suppress cellular immunity is mediated by activating $CD4^+CD25^+$forkhead box protein 3 ($FoxP3^+$) regulatory T cells (Tregs). Tregs have been identified as crucial regulators of cell mediated immune responses[22,54]. They are able to suppress pathogenic immune activities, which play an important role in the context of autoimmune diseases, transplantation and GVHD. Activation of Tregs by IVIg leads to an increased ability of these regulatory cells to suppress allogeneic T cell proliferation *in vitro*[22,54]. High-dose IVIg treatment was demonstrated to stimulate Tregs and, thus, to enhance their suppressive function in humans. This mechanism is currently suggested to be crucial for IVIg-induced restoring of imbalanced immune homeostasis[55]. Regulatory T cell epitopes (Tregitopes) on IgG have been recently identified to trigger the interaction between Tregs and IVIg[56].

HBV HYPERIMMUNOGLOBULIN AFTER LT

HBV hyperimmunoglobulin and HBV-positive liver recipients

HBV-related liver cirrhosis was initially considered as contraindication for LT, due to high rates of fulminant recurrent hepatitis B and posttransplant mortality[57,58]. The introduction of anti-HBsIg (hepatitis B hyperimmunoglobulin; HBIg) in the early 1990's marked a breakthrough for establishing HBV-related liver cirrhosis as standard indication for LT[59].

HBIg is a polyclonal antibody to HBsAg, derived from pooled human plasma[60,61]. It is supposed to bind and to neutralize HBsAg expressing virions. Furthermore, it may prevent cell-to-cell infection within the liver and destruct infected hepatocytes *via* cell-mediated immunity[61]. However, it has only little impact on viral replication. Besides producing significant costs, long-term HBIg monotherapy may promote the development of viral mutations[62,63]. Therefore, a combination of HBIg with potent nucleos(t)ide analogues (NA) is considered as gold standard in prophylaxis of recurrent HBV[62-67].

Currently, the combination of anti-HBs Ig with tenofovir or entecavir is under clinical evaluation[62,68]. These novel drugs are characterized by higher antiviral potency than lamivudine (Lam) or adefovir and, thus, decrease the risk of viral resistances[68]. In combination with high costs and inconvenience of HBIg treatment, strategies of HBIg minimization/withdrawal or even anti-HBs Ig-free prophylaxis may be reasonable. Small sample sizes, short follow-up periods, different virologic risk profiles and inconsistent definitions of viral relapse were major limitations of previous studies. In addition, most trials were predominantly focusing on virologic outcome results, but not on survival data. It became, however, evident in recent years that, with availability of very effective antimicrobial agents, recurrent viral disease no longer reduces patients' long-term prognosis[58,60,68-79]. In order to appropriately assess the prognostic value of HBIg, the focus should, thus, be rather turned on variables like organ acceptance, graft rejection, infectious complications and survival[21,22].

Despite an obvious lack of randomized controlled trials, several clinical studies have in the past demonstrated beneficial immuno-regulatory properties by HBIg which are beyond its antiviral efficacies (Table 1).

Already in 1996, Farges *et al*[80] noticed that 116 HBV-positive liver transplant patients were on a lower risk for acute and chronic graft rejection compared to patients with other indications ($P < 0.05$). Since the immunologic benefit was not paid with an increased risk of infections, these data obviously indicated beneficial immuno-balancing capabilities of HBIg[67]. In contrast, the risk of bacterial infections was significantly higher in 21 patients with alcoholic liver cirrhosis ($P < 0.05$), although their immunologic outcome was comparable to that of HBV-positive liver recipients (Table 1). Apart from that, the incidence of death or retransplantation from rejection or either sepsis or de novo malignancies was significantly lower in HBV-positive liver recipients (3.5%) compared to patients with alcoholic liver cirrhosis (19%; $P < 0.05$; Table 1).

A Brazilian group reported in 2001 on less acute rejection episodes ($P < 0.05$) in 12 HBV-positive liver recipients following long-term HBIg treatment (rejection rate 25%; Table 1) compared to both, HBsAg-positive patients without HBIg treatment ($n = 10$; rejection rate 70%) and HBV-naïve liver recipients ($n = 238$; rejection

Table 1 Clinical data of prognostic relevant immune modulation by hepatitis B hyperimmunoglobulin after liver transplantation

Ref.	No. of patients receiving HBIg	Efficacy of HBIg on immunology/survival
Farges *et al*[80]	n = 116	Significant reduction (P < 0.05) of acute and chronic rejection rate (1.7%) compared to other indications like PBC (6.1%), PSC (13%), AIC (17%), and HCV (9.2%), without increased risk of bacterial infection; significantly lower risk (P < 0.05) of death or retransplantation from rejection or either sepsis or de novo malignancy (3.5%) compared to patients with alcoholic cirrhosis (19%)
Couto *et al*[81]	n = 12	Significantly less acute rejection episodes (0.3 ± 0.5) as compared to HBsAg-positive (0.9 ± 0.7; P = 0.02) and HBsAg-naïve (0.7 ± 0.7; P = 0.03) liver transplant patients without HBIg therapy
Kwekkeboom *et al*[82]	n = 40	Sigificantly lower rate of acute rejection (12%) as compared to patients without viral hepatitis (34%; P = 0.012); only HBIg treatment (HR = 0.39, 95%CI: 0.16-0.99, P = 0.047) and year of LT (HR = 0.87, 95%CI: 0.78-0.98, P = 0.017) were identified as independent predictors of acute rejection
Wang *et al*[83]	n = 1000	Reduction of HBV recurrence rate and of viral mutants; significantly improved 1-yr (P = 0.03) and 3-yr survival (P = 0.005) as compared to an antiviral prophylaxis without HBIg

HBIg: Hepatitis B hyperimmunoglobulin; HBV: Hepatitis B virus; HCV: Hepatitis C virus; LT: Liver transplantation; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cirrhosis; AIC: Autoimmune cirrhosis; HBsAg: Hepatitis B surface antigen.

Table 2 Clinical data of prognostic relevant immune modulation by hepatitis B hyperimmunoglobulin in recipients of hepatitis B virus-positive liver allografts

Ref.	HBV characteristics donor/recipient	Antiviral prophylaxis	Impact of HBIg on outcome
Brock *et al*[94]	HBc+/HBsAg- (n = 958)	HBIg alone: n = 61 HBIg + Lam: n = 66 Lam alone: n = 116 None: n = 509 Missing data: n = 206	70% reduction in risk of mortality by HBIg prophylaxis; (HR = 0.29, 95%CI: 0.10-0.86, P = 0.026)
Li *et al*[112]	HBsAg+/ HBsAg- (n = 63) HBsAg+/HBsAg+ (n = 15)	With HBIg: n = 17 Without HBIg: n = 61 With Lam: n = 14 Without Lam: n = 64	HBIg independently associated with superior posttransplant graft survival; (HR = 0.23, 95%CI: 0.06-0.81) and patient survival (HR = 0.16, 95%CI: 0.04-0.759)

HBV: Hepatitis B virus; HBIg: Hepatitis B hyperimmunoglobulin; HBc+: Hepatitis B core +; HBsAg: Hepatitis B surface antigen.

rate 56%), respectively[81].

In 2005, Kwekkeboom *et al*[82] suggested beneficial immuno-regulatory capabilities of anti-HBsIg. In their study, 40 HBsAg-positive liver transplant patients had a significantly lower incidence of acute rejection (12%) compared with 147 liver recipients without viral diseases (34%; P = 0.012; Table 1). In multivariate analysis, only treatment with HBIg and the year of transplantation were identified as independent factors for reduced risk of acute rejection[82].

And recently, Wang *et al*[83] reported on results of a meta-analysis including 19 studies and 1484 HBV-positive liver transplant patients. Treatment with HBIg was not only associated with reduced risk of viral relapse and development of mutants but lead to a significantly better overall patients' 1- (P = 0.03) and 3-year (P = 0.005; Table 1) survival compared to HBIg-free antiviral prophylaxis. The authors did, however, not find a benefit of HBIg on long-term survival[83].

HBIg and HBV-positive donor livers

The worsening scarcity of donor organs recently prompted several transplant centers to accept donor livers with pre-existing exposition to HBV[15,16]. Particularly anti-HBc-positive/HbsAg-negative donor grafts were increasingly accepted, mainly for HBsAg-positive patients who per se were requiring lifelong antiviral prophylaxis[84-87]. Currently, there seems to be growing need to allocate Hbc+ livers also to HBsAg- patients, especially to those with progressive liver function deterioration or advancing HCC[3,15,16]. In the absence of antiviral prophylaxis, incidences of viral reactivation up to 13%[85-87] and *de novo* HBV infection rates up to 100%[88-95] have been reported. There is currently no standard recommendation for antiviral prophylaxis in this special transplant matching, mainly since well designed studies are lacking. Treatment with HBIg either as monotherapy or in combination with Lam was demonstrated to significantly lower the risk of viral re-activation and infection[96-102]. Recently, monotherapy with potent NAs instead of an HBIg containing antiviral prophylaxis has been proposed[55,99,103]. There are only few trials that have focused on immuno-modulatory impact of HBIg in this special transplant setting (Table 2).

Saab *et al*[102] reported in 2003 on the UCLA experience with 22 HBc+/HCV+ liver allografts. They noted a significant survival benefit in recipients who received a combination of HBIg and Lam compared with those receiving either therapy alone or none of them. However, sample size was rather small and analysis was mixed up with data of other subpopulations[102].

Brock *et al*[94] have specifically addressed this issue in 958 HBsAg-negative liver recipients who received HBc+ liver allografts. Evaluating the UNOS STAR registry data

set, they reported on a 75%-80% risk reduction in graft failure and mortality in HBIg treatment-only compared to Lam therapy-only ($P < 0.001$). Furthermore, improved graft survival was observed for HBIg *vs* Lam-only recipients (HR = 0.34, 95%CI: 0.07-1.56), though data was not statistically significant (Table 2). No allograft failures in this series were attributed to *de novo* hepatitis B infection. Therefore, the authors drew the conclusion that patient and graft survival benefits were rather resulting from anti-inflammatory and immunomodulatory properties than from antiviral efficacies of HBIg treatment[94].

To further expand the available donor pool, the focus recently shifted towards a greater use of HBsAg-positive liver grafts from donors with overt HBV infection, but with normal graft morphology and liver function. Current experiences with these high-risk ECD allografts are still limited, particularly because allocation of HBsAg+ liver grafts is rejected in many transplant centers[104-113]. Therefore, the prognostic value of HBIg and its immunomodulatory efficacies in this special transplant setting is still undefined.

Just recently, however, Li *et al*[112] reported on outcome results of the so far largest series including 78 patients who received HBsAg-positive grafts. By using the US Scientific Registry of Transplant Recipients database, the authors performed a matched analysis and demonstrated comparable long-term patient and graft survival rates between recipients of HBsAg+ ($n = 78$) and those receiving HBsAg- ($n = 312$) liver grafts. Posttransplant outcome of recipients of HBsAg+ livers was significantly better after HBIg prophylaxis as compared to no HBIg treatment (92% *vs* 65% at 5 years for patient survival, $P = 0.01$; 87% *vs* 60% at 5 years for graft survival, $P = 0.02$). In contrast, patient death and graft loss were unrelated to Lam treatment. In multivariate analysis, only the administration of anti-HBs Ig predicted independently posttransplant patient and graft survival in these high risk patients (Table 2).

CYTOMEGALOVIRUS HYPERIMMUNOGLOBULIN AFTER LT

The introduction of specific IVIg against cytomegalovirus (CMV) infection about two decades ago resulted in a significant reduction of viral infection rates posttransplantation[114]. Important developments have since changed the perspectives of CMV infection, like assessment of specific donor (D)/recipient (R) risk constellations and the introduction of potent antiviral drugs (ganciclovir; valganciclovir). Between 1% and 30% of liver transplant patients are supposed to develop CMV disease in the absence of preventive strategies[115,116].

Indirect virus efficacies were demonstrated to account essentially for CMV-related morbidity and mortality[115,116]. These are mainly triggered by the capability of CMV to adversely modulate the recipients' immune system. The virus was demonstrated to up-regulate alloantigens and to increase the risk of acute and chronic allograft rejection[115,116]. It may be associated with vanishing bile duct syndrome and ductopenic chronic rejection and, thus, with risk of cholestatic allograft dysfunction[117,118]. Infection of endothelial vascular cells with CMV promotes the risk of hepatic artery thrombosis and subsequent liver allograft failure[119,120]. In addition, CMV-induced immunologic imbalance increases the susceptibility for other opportunistic fungal and bacterial infections. Apart from that, risk of allograft fibrosis and inflammation may be enhanced and incidence of metabolic disorders was shown to be increased by CMV infection[115,116,120].

Looking at this harmful impact of CMV on the immune system, immuno-modulatory properties by CMVIg could be particularly useful for patients with an increased immunologic and infectious risk profile[21,22]. However, treatment with anti-CMV Ig is currently not a recommended standard in liver transplant recipients[115]. Although well-designed studies on this issue are rare, some larger clinical trials have in the past suggested favourable immuno-balancing capabilities of CMVIg, which are beyond its established antiviral efficacies[80,119-126] (Table 3).

Farges *et al*[80] reported already in 1996 on a significantly reduced incidence of acute rejection in liver recipients who received a 3-mo course of CMVIg (19%) compared to those who did not (48%; $P = 0.01$). Treatment with anti-CMV Ig had no impact on chronic rejection, possibly due to a limited application period. Incidence or severity of bacterial infections was not influenced by treatment with CMVIg[80].

Falagas *et al*[121] demonstrated in 1997 the results of their double-blinded, placebo-controlled CMVIg prophylaxis trial (CMVIg $n = 90$ *vs* Placebo $n = 72$). They reported on a significantly better 1-year survival (86% *vs* 72%; $P = 0.029$) and an obvious trend toward improved long-term survival (68% *vs* 54%; $P = 0.055$) in the CMVIg-population. Furthermore, treatment with anti-CMV Ig was identified as independent predictor of beneficial outcome at one year post-LT in multivariate analysis ($P = 0.042$), and a trend toward increased long-term survival ($P = 0.098$) was also shown[121].

In a meta-analysis including 11 randomized controlled trials, Bonaros *et al*[122] reported about improved overall survival [RR = 0.67 (95%CI: 0.47-0.95)] and reduced CMV-related death [RR = 0.45 (95%CI: 0.24-0.84) after prophylactic administration of CMVIg in solid organ transplantation. However, in the all-cause death analysis, only one liver transplant study has been included[122].

Kwekkeboom *et al*[82] did not observe an outcome benefit by CMVIg in 18 liver transplant patients, which was contrary to their experiences with HBIg treatment. Just recently, differences in the manufacturing process were identified to account for discrepant immunoregulatory capabilities between both Ig-preparations described[123]. The newly manufactured CMVIg was subsequently shown to provide immuno-modulatory capabilities that were comparable to that of HBIg[123].

Two large registry studies recently added data that

Table 3 Clinical data of prognostic relevant immune modulation by cytomegalovirus immune globulin after liver transplantation

Ref.	No. of patients receiving CMVIg	Efficacy of CMVIg on immunology/survival
Farges *et al*[80]	n = 19	Significant reduction of acute rejection rate (19%) compared to recipients without CMVIg (48%; P = 0.01); no impact of on incidence of chronic rejection and bacterial infections
Falagas *et al*[121]	n = 90	Improved 1-yr survival (86% *vs* 72%; P = 0.029) and a clear trend towards improved long-term survival (68% *vs* 54%; P = 0.055). CMVIg as independent predictor of beneficial outcome at one year post-LT (P = 0.042)
Bucuvalas *et al*[124]	n = 336	Lower rate of acute rejection at 3-mo (31% *vs* 40%; P = 0.02); (CMV)Ig treatment as independent predictor for absence of acute rejection (HR = 0.73; P = 0.0019); significantly increased death-free allograft survival (HR = 0.57; P = 0.014) by (CMV)Ig
Fisher *et al*[125]	n = 2805	Significantly lower risk of graft loss and recipients' death (with or without additional antiviral agents; P < 0.001) at 7 yr post-LT; significantly higher 7-yr-survival rate after CMVIg monoprophylaxis (72%) *vs* no prophylaxis (67%; P = 0.02)

CMVIg: Cytomegalovirus immune globulin; LT: Liver transplantation.

emphasized on beneficial immuno-modulatory efficacies of anti-CMVIg[113,114]. Using the Studies of Pediatric LT Registry, Bucuvalas *et al*[124] performed a comparative trial on 336 pediatric liver transplant patients who received either CMVIg or unspecific IVIg for the first week post-LT and 1612 pediatric liver recipients who did not receive any of them[124]. While overall patient survival was comparable between both groups, death-free allograft survival was significantly better in patients treated with (specific or unspecific) Ig (HR = 0.57; P = 0.014). The risk of allograft rejection at 3 mo was 31% for patients receiving, but 40% for those without Ig administration (HR = 0.81, P = 0.029), respectively. The proportion of patients with 2 or more episodes of liver rejection was significantly lower in patients receiving Ig treatment (13.1% *vs* 19.2%; P = 0.009). In multivariate analysis, treatment with IVIg was identified as an independent predictor for absence of allograft rejection (HR = 0.73; P = 0.0019)[124].

Fisher *et al*[125] reported in 2012 on the so far largest study in this special context. Using data of the Scientific Registry of Transplant Recipients, a total of 64.252 liver transplant patients were analyzed, with 2805 of them receiving CMVIg post-LT[125]. The administration of anti-CMVIg (with or without additional antiviral therapy) was associated with lower rates of graft loss and recipients' death at 7 years post-LT (P < 0.0019). Apart from that, CMVIg prophylaxis alone (n = 4559) resulted in a significantly higher survival rate at 7 years post-LT (72%) compared to no antiviral prophylaxis (n = 28508; 67%; P = 0.02), which emphasized on beneficial immune regulation by CMVIg[125].

IVIG AND LT ACROSS IMMUNOLOGIC BARRIERS

Without effective down-regulation of the immune system, transplantation across immunologic barriers may result in hyperacute and antibody-mediated rejection (AMR) and, thus, in organ loss and patients' death. Immunologic incompatibility was, therefore, originally considered as a contraindication in all organ transplants, except LT[127-134]. A positive T-lymphocytotoxic crossmatch, presence of preformed donor specific HLA antibodies and ABO blood group incompatibility are considered as prognostically relevant immunologic barriers in the transplant setting[127,128]. In times of an escalating organ scarcity there is, however, increasing need to accept immunologic incompatible ECD liver allografts[15,16]. Therefore, the issue of implementing immuno-modulatory protocols has gained clinical relevance in recent years[127].

IVIg and LT with positive T-lymphocytotoxic crossmatch

A positive lymphocytotoxic crossmatch indicates the presence of donor-specific antibodies directed either against class Ⅰ or class Ⅱ HLA[127,128]. Increased immunologic sensitization may result from pregnancy, transfusion or previous transplants[127]. The implementation of immune modulation protocols including high doses of IVIg, plasmapheresis and potent immunosuppressive drugs resulted in attenuation of the humoral alloimmune response and in acceptable outcome in highly sensitized kidney and heart transplants[127-133].

The prognostic relevance of a positive T-lymphocytotoxic crossmatch in LT is, however, still discussed controversially[127,134-144]. Originally, the liver was considered to be less susceptible to immunologic attacks. Therefore, pretransplant T-cell crossmatch was either not required, or did not affect the indication for LT[127]. Nowadays, there is increasing evidence that a highly positive lymphocytotoxic crossmatch promotes the risk of acute and chronic allograft rejection, cholestatic liver dysfunction and impaired allograft and patient survival[141-147]. Direct clinical implications of the organ shortage, like pre-LT rising transfusion need, prolonged waiting times and increasing MELD scores, have shown to promote the risk of immunologic imbalance[127,134,148]. This could be one explanation for the reported outcome deterioration in the MELD era[5,10-14]. As a consequence, pre-LT immunologic screening has been recently recommended, particularly in high-risk liver patients[137,138,149].

The prognostic importance of IVIg in highly sensitized liver transplant recipients is currently undefined, since comparative trials are still lacking. In some smaller

Table 4 Clinical data of immune modulation by intravenous immunoglobulins in liver transplant recipients with positive lymphocytotoxic crossmatch

Ref.	Transplant procedure	No. of patients receiving IVIg (pre-LT/post-LT)	Additional immune modulation	Efficacy of IVIg on outcome
Watson *et al*[150]	LT	*n* = 1; post-LT, after detection of AMR	Plasmapheresis, rituximab	Intermittent decrease of Bili, liver enzymes and DSAs'; no survival
Dar et al[151]	SLKT	*n* = 6; pre- and post-LT desensitization	-	Survival rate 83.3%
Kozlowski *et al*[142]	LT	*n* = 3; post-LT, after detection of AMR	Plasmapheresis, rituximab	Transient decrease of Bili, yGT and DSAs' in 2 patients; survival rate 33.3%
Koch *et al*[153]	SLKT	*n* = 1; post-LT, after liver function deterioration and detection of DSAs'	Splenectomy, plasmapheresis, bortezomid	Improvement of liver/kidney function; decrease of DSAs'; survived
Shindoh *et al*[154]	LDLT	*n* = 1; post-LT, after decrease of platelet count and increase of attacking IgG	-	Recovery of platelet count; decrease of attacking IgG; survived
Leonard *et al*[137]	LT	*n* = 2; post-LT, after liver function deterioration	-	Recovery of allograft function; survival rate 100%
Hong *et al*[155]	LDLT	*n* = 1; post-LT, desensitization	-	Survived

IVIg: Intravenous immunoglobulins; LT: Liver transplantation (full size deceased); SLKT: Simultaneous liver-kidney transplantation; LDLT: Living donor liver transplantation; AMR: Antibody-mediated rejection; DSAs: Donor-specific antibodies.

studies of desensitization or treatment, decreasing levels of cytotoxic antibodies and improved allograft function (Table 4) were reported[142,149-155]. Well-designed studies on this subject are needed.

IVIg and ABO incompatible LT

Early results of LT across the ABO blood type barrier were devastation, due to high rates of hyperacute cellular rejection, AMR, vascular thrombosis and ischemic-type biliary lesions (ITBL). As a consequence, ABO-incompatible (ABO-I) LT was originally considered as contraindication[156-158]. In the last decade, ABO-I living-donor LT (LDLT) was implemented in those Asian countries where patients have no chance of receiving a deceased donor graft[159,160]. The escalating discrepancy between growing waiting lists and available donor organs recently put this transplant approach also into the focus in Western countries, mainly for rescue treatment of liver failure and advanced malignancy[161].

Historically, kidney transplantation first broke the ABO barrier and novel immune modulation protocols containing high doses of specific or unspecific IVIg essentially contributed to this success[162-164]. Since immunologic barriers may be even higher in ABO-I LT, its establishment as clinical routine has been more demanding[165].

The introduction of B-cell depletion by a chimeric anti-CD20 antibody (rituximab) and local graft perfusion of vasoactive substances added significantly to improved allograft acceptance in the early 2000's. However, their combination with established immuno-depressive strategies (plasmapheresis, splenectomy, intensified immunosuppression) resulted frequently in aggressive down-regulation of the immune system and, thus, in increasing risks of life threatening infections and vascular complications[165,166].

More recently, treatment with high doses of IVIg was successfully introduced in ABO-I LT[165,166]. The implementation of beneficial immuno-regulatory properties by IVIg encouraged many transplant groups to perform ABO-I LT without complicating local graft catheterization and/or splenectomy[161,165-177].

Testa *et al*[169] reported in 2008 on survival of 4 of 5 patients at mean of 43 mo after ABO-I LDLT following a combination of pretransplant IVIg, pre- and post-LT plasmapheresis and splenectomy.

In the same year, Urbani *et al*[171] demonstrated excellent outcome in 8 patients after ABO-I LT without any case of acute or chronic rejection by using plasma exchange and IVIg. In contrast, there were 3 cases of AMR (27.3%), 5 cases of acute biopsy-proven rejection (45.4%), 1 case of chronic rejection (9.1%) and 3 cases of ITBL (27.3%) following ABO-I LT in 11 patients without IVIg, respectively. Since plasma exchange was performed in both study groups, these results provided some good evidence on beneficial immuno-modulatory capabilities of IVIg, which were beyond its antibody-depleting properties[171].

Ikegami *et al*[172] reported in 2009 on their novel ABO-I LDLT immuno protocol containing rituximab, IVIg, plasmaexchange and splenectomy, but without local graft perfusion. This immuno-regulatory approach was effective and safe in 4 patients after ABO-I LDLT, who were all alive after 26, 8, 6, and 5 mo, respectively. In contrast, two severe catheter-associated complications were reported in 3 historic patients receiving local graft infusion, including one of them suffering from allograft loss[172].

Mendes *et al*[174] reported on a single center experience of emergency ABO-I LT in 10 patients with severe hepatic failure, immediately leading to death without intervention. Plasmapheresis and IVIg were implemented for immune modulation before and after LT. At a mean follow-up of 19.6 mo post-LT, 5 of these

Table 5 Clinical data of immune modulation by intravenous immunoglobulins in ABO-incompatible liver transplant recipients

Ref.	Transplant procedure	No. of patients receiving IVIg (pre-LT/post-LT)	Additional immune modulation	Efficacy of IVIg on immunology/survival
Morioka *et al*[167]	LDLT	*n* = 2; post-LDLT; treatment of AMR	Plasmapheresis	Normalization of liver function; survived
Urbani *et al*[170]	LT	*n* = 1; post-LT; treatment of AMR	Plasmapheresis	Normalization of liver function; survived
Ikegami *et al*[168]	LDLT	*n* = 1; post-LDLT; treatment of AMR	Rituximab, plasma exchange, splenectomy	Normalization of liver function; survived
Testa *et al*[169]	LDLT	*n* = 5; pre-LDLT	Plasmapheresis, splenectomy	Patient and graft survival 80% at mean of 43 mo post-LDLT
Urbani *et al*[172]	LT	*n* = 8; pre- and post-LT	Plasma exchange	Patient and graft survival 87.5% at 18 mo; no case of acute or chronic rejection, no ITBL
Ikegami *et al*[161]	LDLT	*n* = 4; post-LDLT	Rituximab, plasma exchange, splenectomy	Survival rate 100% (28, 8, 6, 5 mo post-LDLT)
Takeda *et al*[173]	LDLT	*n* = 3; post-LDLT; treatment of AMR	Plasma exchange	Normalization liver function; survived
Mendes *et al*[174]	LT	*n* = 10; pre- and post-LT	Rituximab, plasmapheresis	Survival rate 50%; death mainly related to MOF and sepsis
Kim *et al*[175]	LDLT	*n* = 14; post-LDLT	Rituximab, plasma exchange	Survival 100%; no case of acute or chronic rejection
Lee *et al*[176]	LDLT	*n* = 15; post-LT	Rituximab, plasma exchange	Survival 100%; no case of bacterial or fungal infection; 3 cases of biliary strictures
Shen *et al*[177]	LT	*n* = 35; pre- and post-LT	Rituximab	Survival rate 83.1% at 3-yr; one case of acute celluar rejection; two cases of AMR

IVIg: Intravenous immunoglobulins; LT: Liver transplantation (full size deceased); AMR: Antibody-mediated rejection; LDLT: Living donor liver transplantation; MOF: Multi organ failure; ITBL: Ischemic-type biliary lesions.

high-risk liver recipients were still alive[174].

Kim *et al*[175] presented in 2014 excellent outcome results in 14 patients after ABO-I LDLT using a simplified protocol. It consisted of pretransplant rituximab and plasma exchange, and post-LT treatment with IVIg, but without splenectomy and local graft perfusion[175]. Neither AMR nor biliary strictures have been reported after a mean follow-up of 16.2 ± 9.4 mo[175].

Lee *et al*[176] reported on 15 patients after ABO-I LDLT by using rituximab, plasma exchange and IVIg, but without local graft infusion and splenectomy. They demonstrated excellent survival without any case of hyperacute rejection or AMR. Furthermore, the authors did not observe any case of prognostic relevant bacterial or fungal infection[176].

And just recently, Shen *et al*[177] presented their results of a study comparing outcome between emergency ABO-compatible (*n* = 66) and ABO-I LT (*n* = 35). They have adopted a very simplified protocol, consisting of a single dose rituximab and of IVIg at the beginning of LT and for 10 d post-LT, respectively. Plasma exchange, splenectomy and local graft perfusion were not implemented[177]. The 3-year survival rates in these high-risk patients were excellent (86.3% *vs* 83.1%) and rates of complications were comparable between both subsets[177].

Large comparative trials on this issue are not yet available, mainly since ABO-I LT is a highly demanding and very exclusive procedure. Apart from that, the interpretation of previous studies are hampered by differences regarding indications, transplant techniques, recipients' characteristics, immunosuppressive treatments and immune modulation protocols (Table 5). Nonetheless, current available data suggest that the implementation of IVIg and its immuno-modulatory properties contributed significantly to recent outcome improvement in ABO-I LT[165-177].

CONCLUSION

There is increasing experimental and clinical body of evidence that IVIg provides beneficial immuno-modulatory capabilities beyond its antibody-mediated mechanisms. The combination of immuno-stimulating and immuno-suppressive efficacies might be particularly attractive for liver transplant patients with increased infectious and immunologic risk profiles. Although number of immuno-compromised liver recipients was continuously increasing in recent years, well-designed studies on this subject are still rare. Only treatment with specific anti-HBs Ig in HBV-positive liver transplant patients is a recommended standard, but mainly due to its antiviral potency and less for its immuno-regulatory properties.

Current available clinical data on valuable immuno-balancing efficacies of IVIg is intriguing and encouraging, but still based on smaller monocentric studies, larger retrospective registry data and on different outcome variables. However, particularly the identified data on specific IVIg suggest that immuno-modulatory approaches with hyperimmunoglobulins may become more important in times of an escalating organ shortage and its negative clinical consequences. At the very least, they should prompt discussion and emphasize the need to conduct larger prospective trials. It would be very important that future investigations include appropriate risk stratifications, in order to identify subsets that particularly benefit from IVIg. Apart from that, adequate

cost-benefit analyses are needed, since treatment with IVIg may be a rather expensive treatment.

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REVIEW

Current developments in pediatric liver transplantation

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Abstract

In 1953, the pioneer of human orthotopic liver transplantation (LT), Thomas E Starzl, was the first to attempt an orthotopic liver transplant into a 3 years old patient suffering from biliary atresia. Thus, the first LT in humans was attempted in a disease, which, up until today, remains the main indication for pediatric LT (pLT). During the last sixty years, refinements in diagnostics and surgical technique, the introduction of new immunosuppressive medications and improvements in perioperative pediatric care have established LT as routine procedure for childhood acute and chronic liver failure as well as inherited liver diseases. In contrast to adult recipients, pLT differs greatly in indications for LT, allocation practice, surgical technique, immunosuppression and post-operative life-long aftercare. Many aspects are focus of ongoing preclinical and clinical research. The present review gives an overview of current developments and the clinical outcome of pLT, with a focus on alternatives to full-size deceased-donor organ transplantation.

Key words: Pediatric liver transplantation; Deceased organ donation; Living donor liver transplantation; Split liver transplantation; Biliary atresia

Core tip: As of today, pediatric liver transplantation (pLT) has become a safe and routine procedure for the treatment of childhood acute and chronic liver failure as well as inherited liver diseases. In contrast to adult recipients, pLT differs greatly in indications for LT, allocation practice, surgical technique, immunosuppression and post-operative life-long aftercare. Long-term survival after pLT implies life-long aftercare in an interdisciplinary team. The present review gives an insight into current indications for pLT, outcome after living-donor and deceased-donor organ transplantation and of ongoing clinical and preclinical developments to improve long-term outcome.

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INTRODUCTION

In 1953, the pioneer of human orthotopic liver transplantation (LT), Thomas E Starzl, was the first to attempt an orthotopic liver transplant into a 3 years old patient suffering from biliary atresia[1]. LT is the only curative treatment option for patients with irrevocable acute or chronic liver failure and, in the last six decades, has developed from an experimental approach with very high mortality to an almost routine procedure with good short and long-term survival rates. In the early years, long-term survival rates after pediatric LT (pLT) were 11%-39%[2-4] and, since then, have improved to up to 90% with long-term graft survival rates of > 80% (Figure 1)[5,6]. Due to continuing improvements of surgical and interventional techniques as well as perioperative neonatal and pediatric intensive care medicine, the average age of pediatric transplant recipients has steadily declined, with a continuous increase of patients transplanted within the first year of life. As of today, approximately 27% of pLT are performed in recipients younger than 12 mo (Figure 2). Patients in this young age, which in former years could not be transplanted (and mostly died before reaching the size and age of transplantability), today show a long-term survival of almost 90%, which is comparable to older children (Figure 3). At the same time, long-term survival after pLT implies life-long aftercare in an interdisciplinary team to ensure a life with as little as possible secondary morbidity. The present review gives an insight into current indications for pLT, outcome after living-donor and deceased-donor organ transplantation and of ongoing clinical and preclinical developments to improve long-term outcome after pLT.

INDICATIONS FOR PLT

Indications for LT in pediatric patients are manifold and can be classified into cholestatic disorders, metabolic liver diseases causing liver cirrhosis, metabolic liver diseases without liver cirrhosis, acute liver failure, acute and chronic hepatitis, and liver tumors (Table 1). With approximately 40%, the main indication for pLT is biliary atresia. Thus, the indications for pLT are significantly different to indications in adult LT recipients.

In former years, pLT was only performed in curative intent. Today, pLT is also performed, if life expectancy and/or quality of life can be significantly improved. In patients diagnosed with metabolic liver diseases not resulting in liver cirrhosis, the indication for LT has to be carefully evaluated. LT should be performed if the disease can either be cured or extrahepatic manifestations can be significantly improved. A contraindication in this setting would be advanced stage of irreversible extrahepatic manifestations.

LISTING OF PATIENTS AND ORGAN ALLOCATION

Listing of patients

In patients with chronic liver disease, listing for LT should be performed in case of (1) reduced liver synthesis function (*e.g.*, decreased cholinesterase, decreased factor Ⅴ); (2) portal hypertension with or without gastrointestinal bleeding, severe hypersplenism, and/or refractory ascites; (3) failure to thrive despite adequate nutritional therapy; (4) recurrent cholangitis; (5) development of hepatorenal/hepatopulmonary syndrome; (6) recurrent or persistent hepatic encephalopathy; (7) significantly reduced quality of life; and/or (8) early in metabolic liver diseases resulting in life-threatening conditions[7]. Pre- and perioperative morbidity and nutritional status significantly correlate with long-term survival, morbidity as well as physical and cognitive function after pLT[8-13]. Therefore, accurately timed listing and meticulous pediatric management before and after pLT is crucial for long-term outcome.

In pediatric patients with acute liver failure, listing criteria, as in adults, focus more on acute metabolic and synthetic liver function, including the following criteria: hepatic encephalopathy (> grade 2), factor Ⅴ < 20% without adequate increase after sufficient substitution, hyperbilirubinemia (> 17.5 mg/dL), phosphate level above upper reference and/or significant renal failure[7].

Organ allocation

Due to shortage of deceased-donor organs, different allocation solutions are intensively discussed and permanently adapted. In adult LT, a model for the sickest first policy, the Model of End Stage Liver Disease (MELD), was implemented in the allocation procedure within the United Network for Organ Sharing (UNOS) in 2002 and within the Eurotransplant (ET) network in 2007[14,15]. The MELD allocation system is not applicable to all patient groups, especially not to those who have progressive liver disease but no significant impairment of liver or renal function (*e.g.*, patients with liver tumor, some metabolic and/or inherited diseases as well as patients with primary sclerosing cholangitis). For these patients a special allocation system by an exceptional MELD (eMELD) calculation has been implemented[16].

Center based allocation is in use especially in countries with few transplant centers, *e.g.*, in Australia, United Kingdom, and Austria. Moreover, it is used in parallel to the MELD system for extended criteria donor organs. The advantage of the center-based allocation is that the physicians can match the organ to the patient and therefore enable transplantation in patients not well represented by the MELD allocation system. Yet,

Table 1 Diseases indicating pediatric liver transplantation (modified after[7])

Cholestatic disorders	Extrahepatic biliary atresia
	Intrahepatic biliary hypoplasia (Alagille disease, other)
	Progressive familial intrahepatic cholestasis
	Sclerosing cholangitis (primary, neonatal, secondary)
	Nutritive-toxic cirrhosis
	Caroli disease
	Cholangiodysplasia
	Congenital liver fibrosis
	Langerhans cell histiocytosis
Acute liver failure	
Metabolic, with cirrhosis	Alpha 1-antitrypsin deficiency
	Wilson's disease
	Tyrosinemia
	Galactosemia
	Neonatal hemochromatosis
	Cystic fibrosis
	Glycogenosis type Ⅳ
	Metabolic bile acid dysfunction
	Niemann-Pick's disease
	Gaucher's disease
Metabolic, without cirrhosis	Hyperoxaluria
	Crigler-Najjar syndrome
	Urea cycle disorders
	Familial hypercholesteremia type ⅡA
	Glycogenosis type ⅠA
	Hemophilia type A, type B
	Protein C deficiency
	Wolman's disease
	Organic acidemia
Hepatitis	Hepatitis B
	Hepatitis C
	Hepatitis non-ABC
	Autoimmune hepatitis
	Neonatal hepatitis
Liver tumors	Hepatoblastoma
	Hepatocellular carcinoma
	Fibrolamellar carcinoma
	Hemangioendothelioma
Various	Budd-Chiari syndrome
	Cryptogenic liver cirrhosis
	Infantile copper overload

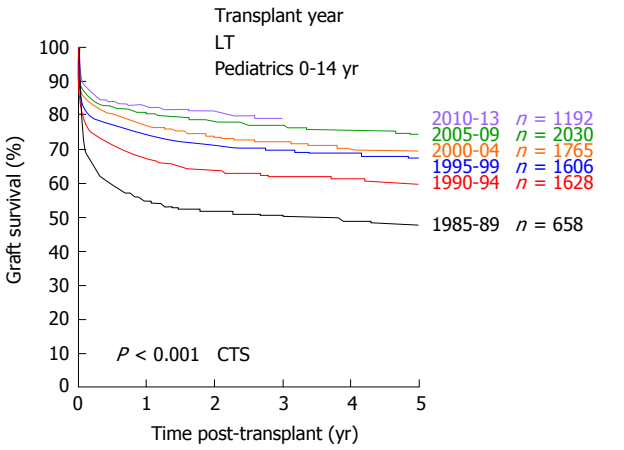

Figure 1 Development of graft survival after pediatric liver transplantation from 1985 until 2013 (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

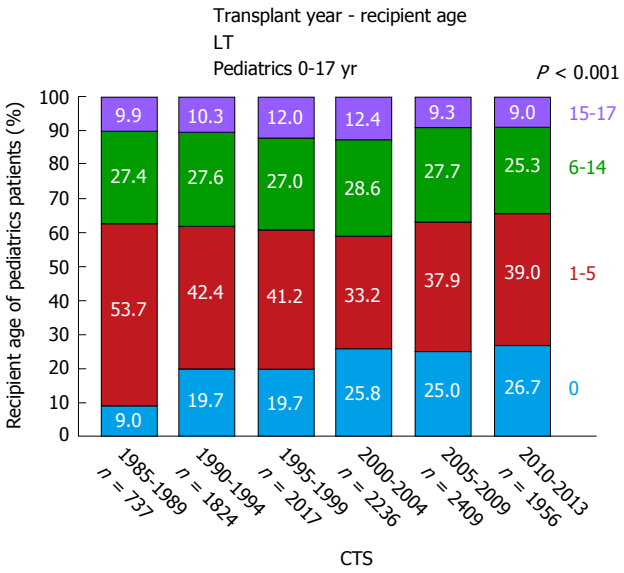

Figure 2 Age distribution of pediatric liver transplantation recipients from 1985 until 2013 (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

this system is prone to a more subjective decision making when allocating an organ and must be assessed critically.

Due to special characteristics in infants and children, especially concerning the inability to develop high serum creatinine values as a marker of severe liver and overall disease, the MELD allocation system can not be applied for this patient group[17,18]. Therefore a special liver allocation system for patients younger than 12 years of age was developed within the UNOS network, not including creatinine as a major component. The so called Pediatric Model for End Stage Liver Disease (PELD) is calculated from serum albumin, bilirubin, INR, age at listing and failure to thrive (based on height, weight and gender) and was implemented for pediatric liver allocation within the UNOS network in 2002[18-20]. Based on multivariate analyses of the Studies of Pediatric LT (SPLIT) database, the PELD score predicts the probability of death or hospitalization to the intensive care unit within 3 mo of listing for LT[19].

When the MELD system was introduced in the ET network in 2007, allocation *via* PELD was not implemented for pediatric liver transplant patients. Alternatively, the so-called matchMELD was introduced, a system comparable to the eMELD granted to defined subgroups of adult recipients not adequately represented by the MELD system. The initial matchMELD at the time of listing is set at a calculated 3-mo-mortality of 35% for children younger than 12 years of age and 15% for children aged 12 to 16 years. Every three months (90 d), the matchMELD increases according to a calculated increase in 3-mo-mortality of 15% (children < 12 years) or 10% (children aged 12 to 16 years). Furthermore, organs derived from small adults or pediatric donors (< 46 kg body weight) are allocated with priority to

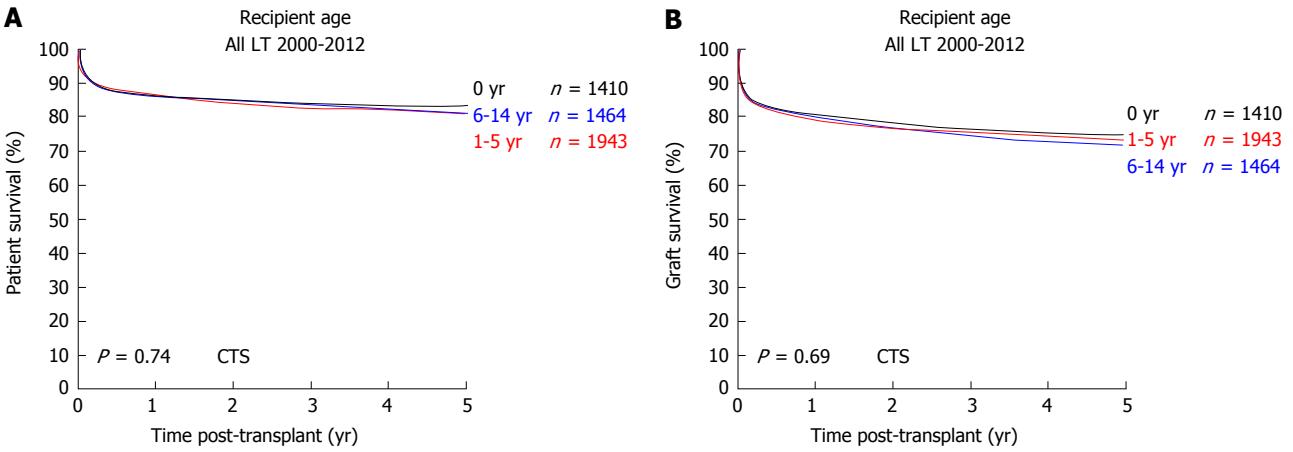

Figure 3 Outcome after pediatric liver transplantation in relation to the recipients age. A: Patient survival; B: Graft survival (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

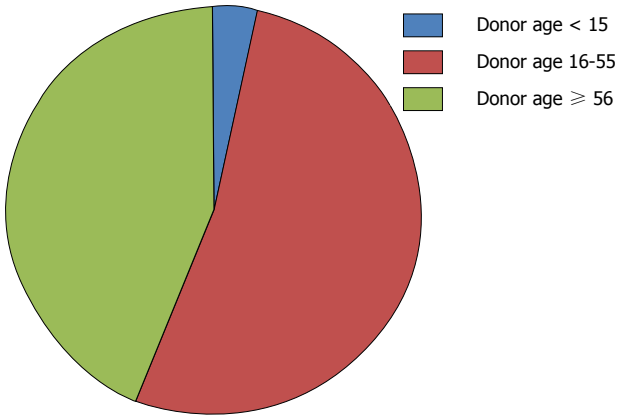

Figure 4 Donor age within the Eurotransplant network in 2013.

pediatric recipients[21,22]. High urgency allocation is generally only possible in case of acute liver failure or for re-transplantation due to graft impairment within 14 d of transplantation.

However, due to a significant mismatch in available pediatric donor organs compared to organs needed for pLT (Figure 4), alternative techniques to increase the donor pool must be applied. Here, living-donor-LT (LDLT) is of particular interest in pLT. In many East-Asian countries deceased-donor liver transplant (DDLT) is rarely performed due to religious and other reasons, which has led to a broad establishment of LDLT in these countries[23-25] and might serve as an example for Western countries to expand the donor pool especially in pLT.

SCARCITY OF DONOR ORGANS AND POTENTIAL SOLUTIONS

Scarcity of donor organs

Before the technique of liver splitting was established, pediatric patients were dependent on donors with similar age or size. In the early 1980's, Christoph Broelsch and Henri Bismuth were the first applying the technique of reduced-size LT in children[26,27]. In 1988, Rudolf Pichlmayr performed the first split LT offering one cadaveric liver to two recipients[28]. However, pediatric deceased donors as well as organs suitable for split-LT remain rare. Figure 4 demonstrates the age of deceased liver donors within the ET network in 2013. Numbers of pLT performed significantly exceed the number of available pediatric organ donors[21].

Surgical techniques: Full-size vs split LT

The technique of full size LT in children is equivalent to adult LT (piggy back or conventional technique). Partial liver grafts can be obtained either by splitting a cadaveric donor organ or by living-donor liver donation. For liver splitting, the anatomical determination of the eight liver segments first described by Couinaud[29,30] in 1957 is essential. Two standard splitting procedures exist: the anatomical splitting (dividing the liver at Cantlie's line) and splitting along the falciform ligament[31]. Splitting of the left lateral segment is technically easier to perform than the true right/left lobe split procedure. Furthermore, the left lateral segment is the smallest part of the liver compared to the extended right, the anatomical left or the right liver lobe and is preferentially used in pLT. In small infants, even the left lateral segment of the liver often is too large and techniques to cut down left lateral lobes may be used to prevent graft-size mismatching and the so-called "large-for-size" syndrome[32]. Due to size mismatch (large graft in small recipient), primary closure of the abdominal wall after pLT is often not possible and should not be enforced in order to prevent compromising graft perfusion by external pressure. In these cases, abdominal wall closure is performed in stages during the first week post-transplant after continuous recovery of the graft from reperfusion injury and edema or accomplished by using mesh grafts[33].

Auxilliary transplantation

A special surgical technique is auxilliary LT [auxilliary partial orthotopic LT (APOLT)] with implantation of a partial graft without fully removing the native liver.

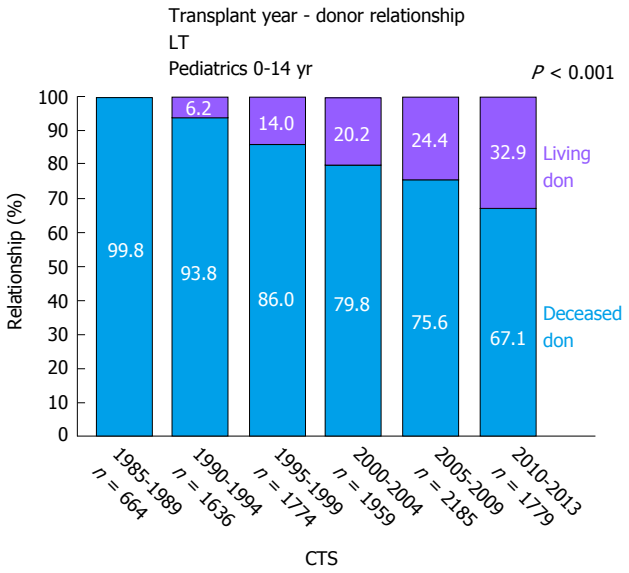

Figure 5 Relation of living (purple) *vs* deceased (blue) donors in pediatric liver transplantation from 1985 until 2013 (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

Gubernatis *et al*[34] reported the first successful case in a patient with acute liver failure. She recovered, her native liver regenerated and immunosuppressive treatment could be withdrawn[34]. APOLT can be successfully performed in children with acute fulminant liver failure or in children with metabolic liver diseases without primary hepatocellular dysfunction or cirrhosis[7,35]. The rationale to perform APOLT in patients with metabolic diseases is to provide sufficient liver mass containing the missing enzyme to correct metabolic function. In case of graft failure, the patient's native liver is still present to secure general liver function. Furthermore these patients preserve the option for later genetic therapy if this can be provided to correct metabolic function in the future[35]. If APOLT is performed in acute fulminant liver failure, *e.g.*, due to severe hepatic necrosis (viral/toxic), the immunosuppressive therapy can be ceased in case the native liver recovers, resulting in an atrophy of the transplanted liver[36]. Yet it must be mentioned that APOLT is technically highly demanding and associated with a higher rate of complications.

Donation after circulatory death

Complementary to splitting organs obtained from donors after brain death, organ donation after circulatory death (DCD) has been shown to increase the organ donor pool. DCD can be performed either as "controlled donation", *i.e.*, planned withdrawal of medical support (ventilation, inotropic support) in the context of catastrophic illness[37], or as "uncontrolled donation" in patients with uncontrolled, out-of-hospital circulatory arrest. Although multiple ethical concerns are connected with donation after circulatory arrest[38,39], the World Health Organization encourages implementation of DCD worldwide[40]. DCD is currently performed in the United States, in 10 of 27 European nations, in Canada, Australia, Japan, China, the Far East and selected South American nations[41].

DCD LT after meticulous donor selection has reached outcomes only mildly inferior to LT after brain death[42], with increased rates of ischemic cholangiopathy and mildly reduced graft survival due to prolonged warm ischemia time[43-45]. Absolute numbers of LT performed after DCD are limited. Within UNOS, pLT after DCD has been performed in 45 cases, compared to 8120 pLT after brain death liver donations from 1996-2012. However, numbers are increasing with 12 transplanted livers (adult and pediatric recipients) from 70 recovered DCD donors in 1996 compared to 2789 transplanted livers from 8297 recovered DCD donors in 2012 within UNOS[41].

Living-donor liver donation

After successful implementation of split-liver LT in pLT, this technique lead to the first LDLT. In 1989, the first series of LDLT in pediatric recipients were performed in Chicago[46]. As of today, LDLT is an established procedure and the main form of LT due to scarcity of deceased donor organs in most East-Asian countries[23]. In western countries and especially in the UNOS area, use of living-donor organs for LT is less frequent and within UNOS constantly < 5% of LT over the last years[47]. Within the ET network, rates of LDLT in pLT are steadily increasing. Analyses of the collaborative transplant study (CTS) database show LDLT rates in pLT of 33% (Figure 5). Retrospective analyses have shown favorable or equal results as compared to pLT after DDLT[48-56]. CTS database analyses show a similar long-term patient survival of pLT after LDLT *vs* DDLT (5-year patient survival 83.7% after LDLT and 81% after DDLT, $P = 0.062$) (Figure 6A). However, long-term graft survival is significantly better after LDLT *vs* DDLT (5-year graft survival 78.2% in LDLT *vs* 71.4% in DDLT, $P < 0.001$) (Figure 6B). The advantages of LDLT are the use of an optimal healthy donor, minimal ischemic time, elective surgery and timing of transplantation according to the recipients' need, which is particularly relevant for pediatric patients, as during a waiting time for pLT, the underlying disease can cause significant somatic and psycho-social long-term morbidity in the developing pediatric organism.

It has been shown that long-term-outcome after pLT significantly correlates with the severity of morbidity at pLT[11]. LDLT offers the possibility and advantage of optimal timing of the transplant procedure before severe morbidity develops. Therefore the main advantage of LDLT is the immediate organ availability for the patient in need. Recipients of living donor livers have a shorter waiting time than recipients of organs from deceased donors. Thus, waiting time mortality can be reduced. However, living donation is not without risk for the healthy donor and LDLT is surgically more demanding than whole organ transplantation. For the donor, major complications (exceeding Clavien grade Ⅱ) were described in up to 44% after right-lobe LDLT and mortality risk was up to 0.8%[57-59]. Right lobe donors

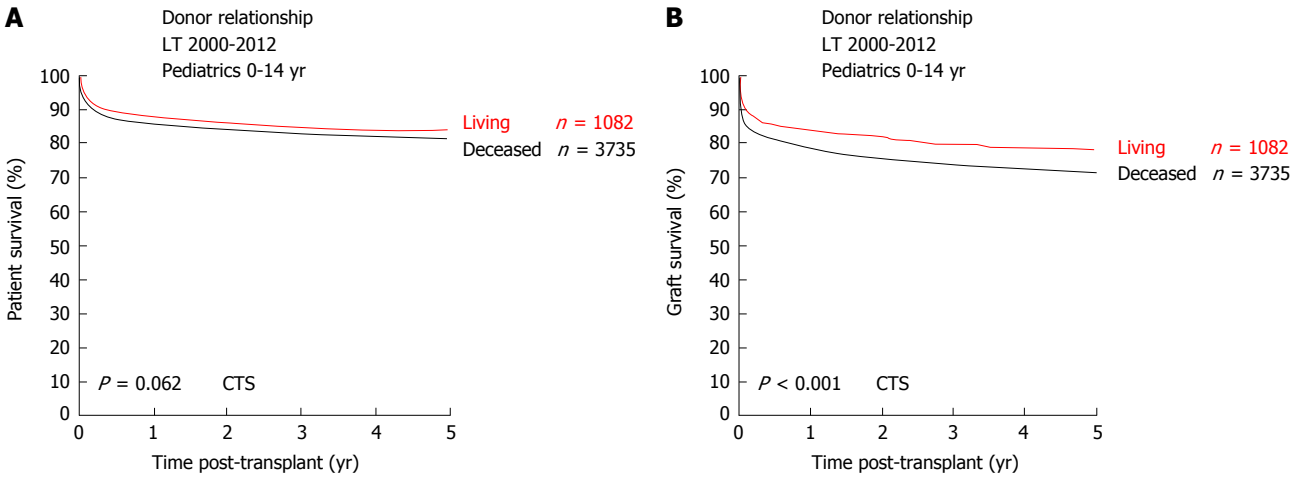

Figure 6 Outcome after living *vs* deceased donor pediatric liver transplantation. A: Patient survival; B: Graft survival (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

undergo operating procedures of longer duration, have significant longer hospital stay and require more blood transfusions[60,61]. However, for pLT, in most cases left-lobe liver donation is performed and the complication rates after full left lobe or left lateral lobectomy are significantly lower[62-64]. Overall biliary complications are one of the major concerns in LDLT donors. In order to decrease morbidity and mortality after liver donation, a thorough evaluation of the potential donor is essential to detect and exclude potential increased medical risk factors for the otherwise healthy donor. Furthermore, complications decrease as surgeon and center experience grows.

OUTCOME AFTER PLT

Age of recipients, patient and graft survival

Analyses of 2192 pLT within UNOS between 1995 and 2006 (1832 DDLT, thereof 1183 whole organs, 261 split organs, 388 reduced size organs; 360 LDLT) showed, that only 33.9% of patients younger than 1 year of age received a full organ, with increasing numbers in older recipients (49.1% in patients 1-5 years of age; 65.3% in patients 5-12 years of age, 79.4% in patients older than 12 years[65]). Operating time, ischemia time and anhepatic time were significantly longer in reduced size or split organs, but with no clinically relevant significance.

Acute graft rejections are observed in 30%-50% during the first year after pLT, but become rare in the long-term outcome. In contrast to adult LT and to transplantation of other organs, acute rejections in pLT do not correlate with long-term outcome or long-term chronic rejection[7,11].

Analyses of the SPLIT database have shown a long-term patient survival after pLT of almost 90%[65], which is in line with CTS database analyses (Figure 3). Mortality in patients > 1 year after pLT is below 5%[66] and mainly caused by posttransplant lymphoproliferative disease (PTLD), recurrent malignancy, sepsis and multi-organ failure. Loss of graft function is observed in 20%-30% after pLT, with < 5% graft loss > 1 year after pLT[66]. In multivariate analyses, predictors of graft loss have been shown to be DDLT split graft, reduced size DDLT graft, fulminant liver failure as indication for pLT, donor age < 5 mo and prolonged warm ischemia time[65].

Acute complications: Comparison of DDLT, LDLT, and split DDLT

Main reasons for patient mortality are early postoperative complications, primary non-function and infections. Reasons for repeated surgical interventions after pLT are complications caused by anatomical-technical aspects. Overall rates of complications are observed in 45.1% after full organ pLT, *vs* 51.9% in LDLT pLT, *vs* 66.7% in DDLT split organ pLT. Repeated surgery within the first 3 mo after pLT is performed in 29.5% after full organ pLT *vs* 41.9% after LDLT pLT and 47.1% after DDLT split pLT. Biliary complications have been observed in 7.5% after full-organ DDLT pLT, which was significantly lower than after DDLT split organ pLT (18.8%) or LDLT pLT (17.5%)[65]. In overall vascular complications and arterial thrombosis, no significant difference was seen between full organ DDLT, split organ DDLT, and LDLT. Portal vein thrombosis has been shown to be significantly lower in full-organ pLT (3.6%) *vs* split DDLT (14.6%) or LDLT (11.1%). Although overall complications, biliary complications and portal vein thrombosis happen significantly more often after LDLT *vs* DDLT, there is no significant difference in 30-d post-LT mortality after full-organ pLT (3%) *vs* LDLT (3.6%), but significantly less in both techniques compared to DDLT split organ pLT (6.9%)[65].

Long-term transplant-related complications

Graft fibrosis has been described in 60% of patients at 10 years after pLT and has been shown to correlate significantly with (1) partial organ graft; (2) young age of recipient; (3) increased donor/recipient age mismatch; and (4) prolonged cold ischemia time[67]. Additionally, graft fibrosis seems to be associated with

high *de novo* donor specific antibodies[67,68]. Comparing graft fibrosis after LDLT *vs* DDLT, no significant difference has been described.

Acute rejections are responsible for 10% of late organ losses[66]. Another 10% of late organ losses are caused by arterial thrombosis and biliary complications[7].

Chronic rejection, even if rare in absolute numbers, develops in 5%-10% of patients and is responsible for 30% of late graft failures[69]. Positive predictors for late graft failure are (1) pLT for malignant disease; (2) pLT for acute liver failure; (3) repeated surgery within the first 30 d after pLT (other than scheduled 2nd look surgery); (4) > 5 hospital admissions during the first year after pLT; and (5) steroid-resistant acute rejections[66].

Long-term morbidity and quality of life

In addition to direct transplant-related complications, long-term morbidity and quality of life is a main focus in ongoing research in pLT. Major long-term complications after pLT are reduction of kidney function (17%-32% of patients after pLT[70,71], arterial hypertension (15%-30% of patients after LT)[72,73] and development of secondary neoplasias, particularly PTLD (5%-10% of patients after LT)[74,75].

Kidney function can be reduced as a consequence of long-term immunosuppression, but may also be caused by the underlying disease (*e.g.*, Alagille's disease). Furthermore, long-term influence on kidney function of many chronic liver diseases before LT is unknown. Therefore development of kidney protective new immunosuppressive regiments (see below) and close post-pLT aftercare including translation of care into adulthood are crucial for long-term morbidity and quality of life.

PTLD is seen in up to 15% of patients after pLT and mortality rates of 30%, in single reports of up to 50% have been described[76,77]. Main risk-factors for the development of PTLD are Epstein-Barr virus-naïve recipients, high total immuosuppressive load and the intensity of active viral load[78]. In addition to optimal antiviral therapy, the choice of the immunosuppressive regimen can significantly influence the risk of PTLD and is an ongoing focus of preclinical and clinical research.

Of special importance in pediatric organ transplantation is the problem of achieving a successful transition into adult care. Medication nonadherence as one of the main problems has been described in 17%-53% adolescents after LT[79]. Nonadherence to medical regimens post transplantation increases rates of complications, graft rejection, health care utilization and mortality. Therefore targeting problems of nonadherence should be the main focus in strategies to improve the transition process[80].

IMMUNOSUPPRESSION

Equal to patients after LT from a deceased donor, patients after living liver donation require immunosuppression to avoid immediate as well as long-term rejection of the transplanted organ. Therefore all patients, adults and children, are treated according to standardized immunosuppression protocols consisting of protocols for the early post-transplant period and protocols for long-term maintanance therapy.

As in adult LT, the introduction of calcineurin inhibitors (CNI) in the early 1980s gave way to long-term survival also for pediatric transplant recipients and until today remain the backbone of immunosuppression protocols[81,82]. The early post-transplant phase is the time of highest risk for immunologic reactions between graft and host and therefore the highest immunosuppression is required during this period. Most protocols comprise of induction therapy, dominated by interleukin-2 receptor antibodies especially in the pediatric transplant population (Basiliximab® and Daclizumab®), combined with corticosteroids and calcineurin inhibitors (cyclosporine A and tacrolimus) as maintenance therapy[83-88].

In contrast to adults, the use of other mono- or polyclonal antibodies [*e.g.*, monoclonal anti-CD3 antibody preparations (OKT3) and rabbit or equine antithymocyte globulin] for induction therapy has not been adopted by the pediatric transplantation community because of concern of undesired short - and uncertain long-term effects of such potent drugs on the developing organism and immune system[89].

Over the past years many studies could show that an overall minimization of immunosuppression is possible, especially in pediatric liver transplant patients, which may be of significant advantage for long-term quality of life. Especially in pediatric recipients, it is of great concern to compose the immunosuppressant drugs according to the individual need to minimize long-term undesired side effects[90-93]. The main goal of drug minimization is reduction of negative side effects, especially on the growing organism, and avoiding long-term morbidity while preserving graft function. The most significant side-effects of different immunosuppressants are nephrotoxicity, diabetes, development of hypertension, hyperlipidemia, impairment of growth, neurologic alterations, hypertrichosis and bone marrow suppression. Yet, up to date we are missing appropriate tools to determine the optimal level of immunosuppression due to great differences between individuals as well as within the same individual over time.

Regarding these aspects and based on increasing data to safety aspects in the use of different immunosuppressant drugs in the adult population, multiple combination treatments, such as mycophenolate-mofetil and mammalian target of rapamycin inhibitors (Sirolimus and Everolimus), with and without CNIs have been introduced for maintenance therapy also in pediatric solid organ transplant patients and are topic of ongoing studies[94-101]. By this strategy the single immunosuppressive drugs may be decreased to levels that do not cause significant clinical side-effects but are sufficient to avoid rejection.

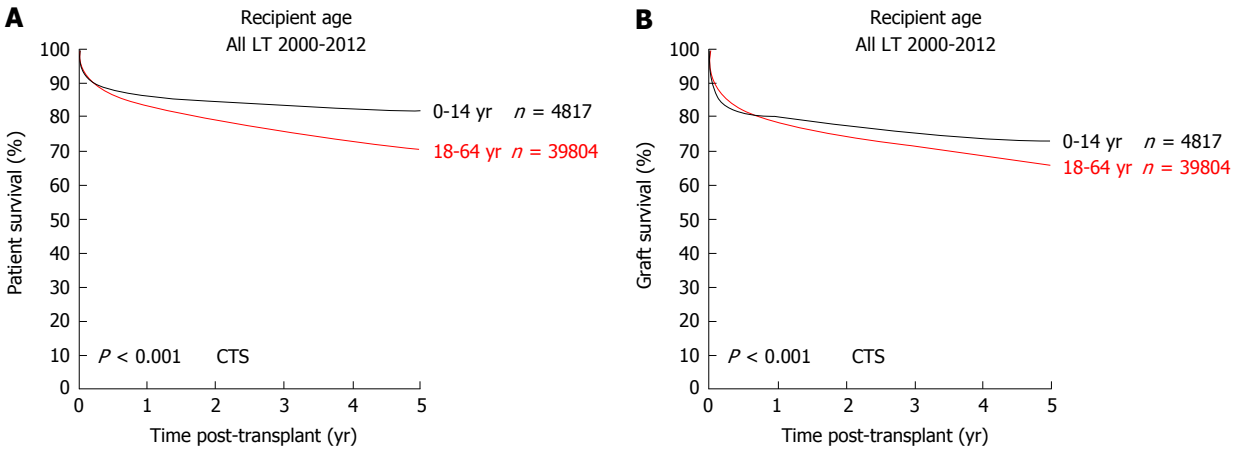

Figure 7 Outcome of liver transplantation in pediatric *vs* adult recipients. A: Patient survival; B: Graft survival (collaborative transplant study data). CTS: Collaborative transplant study; LT: Liver transplants.

IMMUNE TOLERANCE AND WITHDRAWAL OF IMMUNOSUPPRESSION IN PLT RECIPIENTS

Up to date, life-long immunosuppression is suggested after solid organ transplantation, but more and more data is evolving that especially patients who are transplanted early in life or receive a parental living liver donation may develop a certain extent of immune tolerance towards the transplanted graft. Single center experiences in which patients were withdrawn from immunosuppression because of medical reasons (*e.g.*, PTLD or renal insufficiency) or had self-withdrawn their medication due to non-compliance suggest that approximately 20% of liver transplant patients become operationally tolerant towards the graft[102-107]. Yet, up to date there are no reliable markers to determine, which patient has developed tolerance and which patient should remain on immunosuppressive drugs. Clinical experience shows that graft rejection may occur even years after weaning of immunosuppression and a focus of ongoing research is the definition of robust markers for distinguishing tolerant from non-tolerant liver transplant patients[107,108].

Another, more aggressive approach to induce immune tolerance in solid organ transplantation is to combine solid organ transplantation with hematopoietic stem cell transplantation from the same donor[109-111].

In our opinion future immunosuppressive strategies in pLT have to imply 3 main goals: (1) minimization as well as individualization of immunosuppression to reduce long-term negative side effects; (2) preservation of long-term allograft function; and (3) development of strategies to monitor and induce tolerance as well as differentiate between operationally tolerant and non-tolerant patients.

CONCLUSION

pLT is a routine and safe procedure to treat acute or chronic liver failure or selected metabolic liver diseases in children. Short and long-term survival are significantly better in pLT compared to LT in adult recipients (Figure 7) and patient survival curves plateau at 4 years after pLT. A main problem of pLT, especially within the ET network, is the scarcity of pediatric donor organs or organs suitable for splitting after DDLT. Here, LDLT is a valid solution and should further be promoted. In conspect with the comparable long-term patient survival after LDLT and increased graft survival after LDLT *vs* DDLT pLT, results discussed in this review on outcome after pLT lead us to the following conclusions: (1) In pediatric LT, LDLT is a safe procedure with long-term outcomes equal to or even better than DDLT; (2) In small infants, where full-organ LT is not an option due to donor/recipient size mismatch, LDLT enables LT in patients which in former times could not be transplanted due to the scarcity of deceased donor livers suitable for splitting; (3) LDLT enables pLT at a recipient-controlled time, when perioperative morbidity can be minimized and long-term negative effects of the underlying disease may be prevented; (4) Immunosuppression after LDLT can often be significantly reduced in pediatric recipients and further research in immunosuppressive therapies may in future minimize immunosuppression-related morbidity and PTLD and, in some cases, may induce immune tolerance; (5) Microsurgical techniques and interdisciplinary management of pLT recipient need to be further improved to reduce acute complications due to biliary or portal vein complications and to further increase long-term patient and graft survival; (6) In line with the latter argument, pLT should be exclusively performed in highly specialized centers, where several disciplines (pediatric transplant surgery, pediatric and adolescent medicine, pediatric intensive care medicine, interventional radiology and anesthesiology trained in pediatric treatment) closely interact and are on call 24/7/365; and (7) Meticulous donor selection and donor safety must continue to have highest priority in LDLT.

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REVIEW

Management of hepatocellular carcinoma in the elderly

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Abstract

Mean age of hepatocellular carcinoma (HCC) patients has been progressively increasing over the last decades and ageing of these patients is becoming a real challenge in every day clinical practice. Unfortunately, international guidelines on HCC management do not address this problem exhaustively and do not provide any specific recommendation. We carried out a literature search in MEDLINE database for studies reporting on epidemiology, clinical characteristics and treatment outcome of HCC in elderly patients. Available data seem to indicate that in elderly patients the outcome of HCC is mostly influenced by liver function and tumor stage rather than by age and the latter should not influence treatment allocation. Age is not a risk for resection and older patients with resectable HCC and good liver function could gain benefit from surgery. Mild comorbidities do not seem a contraindication for surgery in aged patients. Conversely, major resection in elderly, even when performed in experienced high-volume centres, should be avoided. Both percutaneous ablation and transarterial chemoembolization are not contraindicated in aged patients and safety profile of these procedures is acceptable. Sorafenib is a viable option for advanced HCC in elderly provided that a careful evaluation of concomitant comorbidities, particularly cardiovascular ones, is taken into account. Available data seem to suggest that in either elderly and younger, treatment is a main predictor of outcome. Consequently, a nihilistic attitude of physicians towards under- or no-treatment of aged patients should not be longer justified.

Key words: Hepatocarcinoma; Epidemiology; Cirrhosis; Elderly; Treatment

Core tip: The number of elderly patients with cancer is expected to rise in the next future, and facing with elderly cirrhotic patients with hepatocellular carcinoma (HCC) will characterize liver oncology scenario in the near future. International guidelines do not specifically address how to approach HCC in aged patients and no recommendations are available on age threshold to which clinical decisions should refer. Available data seem to rule out an intrinsic negative impact of age itself on HCC prognosis, and treatment allocation should be decided mainly according to HCC stage, liver residual function and general conditions. Indeed, a nihilistic

attitude to restrict treatment in this population is not longer justified.

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INTRODUCTION

In Western countries, the number of elderly subjects is increasing, and 75 years old people may have an expected life expectancy of 5-10 years[1]. The progressive ageing of population also means that the number of elderly patients with cancer is expected to rise in the next future[2-4]. It is widely accepted that the risk of developing hepatocellular carcinoma (HCC) is age-dependent[5]; hence, in our countries, the diagnosis of HCC is more frequent in patients aged ⩾ 70 years[6]. In fact, over the last two decades, the mean age of HCC patients at first diagnosis has progressively increased from 60 years in the mid-nineties to 70 years in more recent series[6-11]. Thus, facing with elderly cirrhotic patients with HCC is becoming a routine in clinical practice, and clinicians should be aware of the scenario that will characterize liver oncology in the near future. Therefore, investigations on the approach to HCC in aged patients are urgently warranted. International guidelines do not specifically address whether the management and outcomes of HCC in elderly patients are different from those observed in their younger counterpart[12-16]. In fact, elderly patients are usually under-represented in clinical trials or in seminal studies, which represent the key evidence to support the recommendations on HCC management. Therefore, a gap between guidelines and clinical practice may arise. In particular, no recommendations are available on age threshold to which clinical decisions should refer and older patients are merely defined as difficult-to treat or fragile patients[17-19]. Establishing an "a priori" age threshold for HCC treatment could be viewed as unethical; in daily practice, however, age plays a critical role in the decision making process of HCC treatment, with particular reference to liver transplantation and resection.

In this brief review, we will focus on some relevant issues associated with the management of HCC in elderly patients, with the aim of providing physicians with some scientific information useful to approach the management of these patients. An extensive literature search in MEDLINE was performed with different combinations of the following keywords: "hepatocellular carcinoma" AND ["surgery" OR "hepatectomy", OR "resection", OR "radiofrequency", OR "percutaneous ablation", OR "chemoembolization", OR "TACE", OR "radioembolization", OR "sorafenib"] AND "meta-analysis", "randomized controlled trial", "prospective study" or "retrospective study" AND "elderly". We restricted the time interval for literature search regarding overall survival and disease free survival in specific treatment approach from January 2000 to October 2014, because several international guidelines on management of HCC have been produced worldwide in that period. However, we included few articles published before, because of their relevance on general epidemiology and changing population scenario of HCC. In addition, bibliographies of review articles were hand-searched to identify additional relevant studies and randomized controlled trial on therapeutic outcome in elderly, which were considered for analysis. Only articles published in full text and in English language were considered. Abstracts were not included. The title and abstract of studies identified in the search were reviewed by two authors independently (Borzio M and Dionigi E) to exclude studies that did not address the specific research question of interest. After this initial screening, a cross-checked to identify discrepancies was done. If multiple publications from the same cohort were found, the most recent report was considered. The latest electronic search date was the 30 October 2014.

ELDERLY: DEFINITION OF AND CLINICAL IMPLICATION

The concept of "elderly" has become more difficult to define. Definition of elderly is still uneven, mostly because the life expectancy varies from different geographical areas. Therefore, there is no general agreement on the age at which a person should be considered old[20]. Moreover, chronological age is not necessarily a synonymous of biological age, and this latter may be different in men as compared to women. In general, the chronological age of 65 years-roughly equivalent to retirement age - is currently accepted as a threshold to define an "elderly" person. In scientific literature on liver disease, and in particular in papers dealing with HCC, the most used threshold is 70 years[21-23]. More recently, clinical studies adopting a threshold of 75/80 years have been published[24-26].

The increasing age of the HCC population brings some drawbacks to HCC treatment, due to the occurrence of comorbidities which can be associated with reduced treatment tolerability and an increased risk of severe toxicity. In a recent survey on naïve HCC patients by our group, the prevalence of relevant comorbidities in aged patients was > 60%[6]. Comorbidities could also limit the access to proper treatment and may represent a barrier hampering the adherence to therapeutic flow-charts recommendations by international guidelines. Moreover, comorbidities make difficult the correct staging of HCC in elderly patients. According to the classification of the barcelona clinic for liver cancer (BCLC), which is the most widely-adopted staging system worldwide, Eastern Cooperative Oncology Group Performance Status that

quantizes constitutional syndrome due to tumour burden is one of the key variables which determine disease stage and, consequently, influence treatment allocation[27]. In particular, according to BCLC, patients with a PS $\geqslant$ 1 are excluded from curative treatments regardless of tumour extension. However, the difference between PS 0 and 1 is very narrow, and it is based only on the ability to carry out heavy works. It appears intuitive that this ability could deteriorate simply because of ageing and/or presence of comorbidities, and it may be independent from disease stage. This problem has been recently addressed and adjustments of BCLC staging system are warranted[28]. Another problem frequently encountered in elderly patients is the reluctance of relatives in approving to invasive therapies, erroneously considered too risky. In this context, the final therapeutic decision should be taken within a multidisciplinary setting and be shared with relatives who should be made aware of survival benefit and risks of treatment balanced to life expectancy.

EPIDEMIOLOGIC CONSIDERATIONS

Older population with HCC is characterized by a higher prevalence of females[29,30]. This likely simply reflects the longer life span in this gender. The reasons for the higher proportion of females in elderly HCC patients are that the average life expectancy at birth for females is longer than that of males, and thus the proportion of females is higher than that of males in the elderly population.

In many studies, elderly patients with HCC were more likely to be hepatitis C virus (HCV) carriers[30-43]. In fact, unlike HBV infection, most HCV infections are acquired late in life and HCV-related carcinogenesis needs a long-time interval to accomplish. Nonalcoholic steatohepatitis (NASH) is another etiologic condition frequently associated with HCC in elderly. NASH-related carcinogenesis is indeed characterized by a long-lasting and insidious development. Patients with NASH-related HCC are generally older than those with virus-related HCC[44,45]. Given that a relevant proportion of cirrhosis previously classified as cryptogenic are indeed NASH-related[46], it must be argued that a large proportion of non-viral, non-alcoholic HCC in elderly are related to longstanding NASH, in particular when associated with diabetes[44,45].

With respect to the gross pathology of HCC at presentation, several studies reported that in elderly patients HCC is more frequently mono-pauci focal and it is frequently associated with less advanced fibrosis. It is well-known that multifocal liver carcinogenesis is associated with the degree of liver fibrosis while, with ageing, carcinogenesis has much more time to progress even in the absence of relevant inflammation and fibrosis[26,34-42,47-52].

It has been reported that HCC in elderly patients was more frequently encapsulated[35]. It is well known that encapsulation is a favorable prognostic factor for HCC being indicative of higher differentiation of HCC and a lower incidence of vascular invasion[37,38].

HCC OUTCOME IN ELDERLY

Prospective studies specifically designed to compare the outcomes of HCC in older and younger patients are lacking. Available data on HCC outcome in elderly patients mainly derive from retrospective sub-analyses of observational, in-field surveys performed in the last decades and designed to follow HCC patients prospectively. These studies showed that short-term survival was unaffected by age and it was primarily predicted by cancer stage and the underlying cirrhosis[24,29,41]. Conversely, long-term survival in elderly patients is mostly dependent on their expected shorter life-span and occurrence of comorbidities. Kim *et al*[29], in a large series of Korean HCC patients, showed that non-liver related mortality was significantly higher in older patients (> 70 years) than in younger subjects, although the overall survival was similar to that found in non-aged patients.

Available data seem to rule out an intrinsic negative impact of age itself on HCC prognosis, suggesting that treatment allocation should be decided according to HCC stage, liver residual function and general conditions, rather than to age of patients. In addition, hepatic functional reserve in elderly HCC patients was almost the same as that in younger HCC patients.

Consequently, as for younger patients, in older patients the early diagnosis of HCC is mandatory and aged cirrhotic patients should not be excluded from ultrasound screening/surveillance programs. The evidence that elderly patients undergoing treatment displayed a similar survival rate compared with younger patients, and the survival rate depended solely on whether treatment was initiated, further support the role of treatment itself as an independent predictor of outcome irrespective of age[29]. Results from a sub-analysis on data collected from a large cohort of "real-life" Italian cirrhotic patients (CLIP cohort), prospectively followed over a period of twelve years, showed that being under treatment was an independent predictor of better prognosis for elderly patients[41]. This would mean that a nihilistic attitude to restrict treatment does not appear justified in this population.

RESECTION

Hepatic resection is considered a first-line curative therapy for early HCC in patients with well-compensated cirrhosis, and in those with large tumours and without cirrhosis. However, elderly patients have long been considered unfit for surgery due to their intrinsic fragility. Moreover, practical guidelines do not specify any age limit for surgery. In the last decades, however, technical progresses have made surgery for HCC in elderly patients safer and feasible[53]. Studies comparing the outcome of HCC resection in old and young patients

Table 1 Outcome of elderly and younger patients with hepatocellular carcinoma undergoing resection

Ref.	Age limits (yr)	O/Y	Survival (%)			DFS		
			1 yr	3 yr	5 yr	1 yr	3 yr	5 yr
Yeh *et al*[38]	70	30/398	85	64	39	NA	NA	29
			NA	46	NA	NA	NA	27
Zhou *et al*[39]	70	55/124	89	57	50	74	31	30
			80	49	38	63	34	16
Kondo *et al*[34]	70	109/210	78	45	42	NA	NA	NA
			79	52	47			
Kaibori *et al*[50]	70	155/333	NA	70	55	NA	30	21
			NA	70	57	NA	38	23
Oishi *et al*[25]	75	62/504	NA	77	58	NA	43	30
			NA	81	64	NA	46	28
Huang *et al*[35]	70	67/268	83	55	43[b]	69	58	47
			72	40	31	65	41	36
Poon *et al*[23]	70	31/299	79	58	29	57	27	27
			75	51	40	54	38	24
Tsujita *et al*[47]	75	23/77	95	70	NA	60	38	NA
			96	83	NA	61	35	NA
Su *et al*[36]	55	700/374	87	66	51	NA	NA	NA
			82	67	59	NA	NA	NA
Nishikawa *et al*[51]	75	92/206	90	73	43	66	39	26
			91	78	64	66	39	22

[b]$P < 0.01$. Data on younger patients are reported in italics. Only articles reporting on either survival and disease free survival (DFS) were considered. O/Y: Old/young.

performed in the last 15 years indeed documented encouraging results[25,26,34-39,42,47-50] (Table 1).

Many authors agree that age is not a risk factor for resection and those older patients with HCC and good liver functional reserve could gain benefit from surgery. In surgical series, the rate of HCCs diagnosed in the background of non-cirrhotic liver ranged from 0.3% to 30% approximately[37,54]. It cannot be excluded that this bias of selection may have influenced the final favourable outcome in aged patients treated with surgical resection. However, these findings may simply reflect the more scrupulous and restrictive criteria as to liver function reserve adopted in these fragile patients before allocating them to liver surgery.

Opposite conclusions were in two independent studies from different geographical areas, multivariate analysis revealed that age was an independent negative predictor of outcome after liver resection[55,56].

Data on survival and disease free survival in old and young patients undergoing HCC resection are reported in Table 1. Post-operative mortality in elderly patients was reported to range from 0 to 3.2%[25,34-42,48-50,55-57].

In two retrospective studies from Far East, surgery-related in-hospital morbidity and mortality were not significantly different in older as compared to younger patient[26,50]. A surprisingly high mortality (10.5%) was found in aged (> 70 years) resected patients which, however, was not so different from that observed in younger patients (7.7%).

Mild comorbidities do not seem a contraindication for surgery in aged patients. Poon *et al*[23], in their retrospective analysis on aged patients (≥ 70 old) undergoing hepatic resection for HCC, concluded that surgery is safe in well-selected patients even in presence of comorbidities. In the study by Huang *et al*[35], a better post-surgical overall survival was reported in the elderly group despite a higher prevalence of comorbidities.

Conversely, chronic renal diseases and cardiovascular diseases were significantly associated with higher mortality in elderly in Sato *et al*[54] study.

Comorbidities such as cardiovascular diseases or chronic renal diseases should be therefore carefully evaluated before present aged patients as a candidate to hepatic resection, in particular if HCC is associated with cirrhosis.

Comorbidities were among the five characteristics included in a recently proposed simple risk score, to predict in-hospital mortality after hepatectomy for HCC. The strongest predictors of in-hospital death were a Charlson score of 3 or more (indicating at least 2 comorbid conditions or those of greater severity) and a more invasive procedure (lobectomy)[58].

Major resection (> 3 segments) should be considered with caution in older patients. Portolani *et al*[56], in a multivariate analysis on 175 elderly patients undergoing surgery, showed that major resection was an adverse predictor of overall survival (OS). Similar results were reported by Reddy *et al*[40] who found that increasing age (> 60 years) was independently associated with postoperative mortality after major hepatic resection even when performed in experienced, high-volume centres. The authors concluded that major resection in elderly patients should be avoided or limited to selected cases and possibly performed by experienced.

Attitude to perform liver resection in older patients is indeed another variable influencing the outcome. High-volume hospitals seem to be characterized by lesser morbidity and in-hospital mortality[55].

Table 2 Outcome of elderly and younger patients with hepatocellular carcinoma undergoing radiofrequency ablation

Ref.	Age limits (yr)	O/Y	Survival (%)			DFS		
			1 yr	3 yr	5 yr	1 yr	3 yr	5 yr
Tateishi *et al*[62]	68	159/160	NA	76	NA	NA	NA	NA
			NA	79	NA	NA	NA	NA
Mirici-Cappa *et al*[41]	70	159/230	90.1	53.4	29	NA	NA	NA
			89.9	52.9	35.1	NA	NA	NA
Nishikawa *et al*[31]	75	130/238	90	64.1	44.8	66.9	21.3	19
			97.6	83.7	64[b]	80.5	40	19.5[b]
Takahashi *et al*[32]	75	107/354	NA	82	61	NA	49[2]	56[2]
			NA	80	63	NA	49[2]	56[2]
Kao *et al*[33]	65	158/100	NA	NA	81.3	NA	NA	NA
			NA	NA	65.4	NA	NA	NA
Hiraoka *et al*[63]	75	63/143	93	83	50[1]	NA	NA	NA
			93	78	58	NA	NA	NA

Data on younger patients are reported in italics. Only articles reporting on either survival and disease free survival (DFS) or overall recurrence[2] were considered. [b]$P = 0.001$; [1]Not significant. O/Y: Old/young; NA: Not available.

Overall, available data should reassure surgeons and hepatologists on the feasibility of curative resection even in elderly patients. On a patient-by-patient approach, surgery should be offered to well-selected cases following a multidisciplinary discussion and after a careful evaluation of resection benefit, risks of treatment and expected life span.

LIVER TRANSPLANTATION

Orthotopic liver transplant (OLT) is a curative treatment for HCC Due to the organ shortage, access to OLT is still narrowed and age is one of the most important variables limiting access to the waiting list. Living donor transplant (LDT) may be a reliable option even for older patients but LDT programs are still limited or not available in many countries. Although there is not an established age limit for OLT, an arbitrary threshold of > 65-70 years is generally adopted worldwide. Elderly patients are considered poor candidates due to the frequent presence of ischemic heart disease and diabetes, which are known to adversely affect post OLT course.

OLT outcome in patients ⩾ 70 years has been only seldom documented. In the study by Taner *et al*[57], 13 transplanted patients ⩾ 75 years experienced a favorable outcome and seven of them experienced a mean survival of 65 mo. A large-scale survey from Switzerland concluded that advanced age was not a significant predictor of survival[58]. However, in clinical practice, patients older than 65 years are seldom listed for OLT. In countries where paucity of organ donors is a problem, OLT for HCC in elderly remains an unrealistic option.

RADIOFREQUENCY ABLATION

Percutaneous thermal ablation is recommended as a curative treatment for single unresectable HCC < 3 cm or multiple HCC (till 3 nodules < 3 cm). Since its introduction in the early 90's, radiofrequency has gained popularity being, in early HCC, equally effective, safer and less invasive than resection. In a population-based survey carried out in United States which addressed temporal changing of therapeutic interventions to HCC in clinical practice, a 43% increase of RFA over time was found, and this change was particularly evident in aged patients[60].

Studies on outcome after radiofrequency ablation (RFA) in elderly patients yielded conflicting results (Table 2). In two retrospective studies performed on large series from Japan, old age emerged as an independent predictor of poor prognosis at multivariate analysis[61]. In the study by Nishikawa *et al*[26], the reduction in OS observed in elderly patients compared with their younger counterpart was primarily due to the higher rate of early recurrence. Conversely, other studies have reported that old age was not a independent predictor of reduced survival after RFA[60-62]. Good safety profile of RFA is maintained in elderly and the rate of major complications was found to be similar in elderly and in younger cirrhotic patients[32]. Comorbidities seem unlike to impact on the post-RFA outcome.

Overall, RFA as well as other ablative techniques such as percutaneous alcohol injection and microwaves could be used in elderly subjects with satisfactory results and outcome and may represent a valid alternative to surgery for early HCC being less risky and largely preferred in these fragile patients. Moreover, percutaneous ablation is by far the most frequently used treatment for HCC recurrence. Clinicians should be therefore trained in these procedures, particularly when facing with patients aged 75 years or more.

TRANSHEPATIC ARTERIAL CHEMOEMBOIZATION

The efficacy of transarterial chemoembolization (TACE) for unresectable HCC in cirrhotic patients emerged from two meta-analyses published during the last ten years. For these reasons, American and European guidelines

approved TACE as the treatment of choice of HCC in intermediate stage in cirrhotic patient with preserved liver function. There are only few studies addressing the use of TACE in elderly and its use in this setting is still debated. In two retrospective cohort studies, TACE was less frequently offered to older patients mainly because this technique was considered less feasible and potentially risky in elderly patients[24,41]. More recent studies did not confirm this finding. A prospective cohort study performed on 102 patients with HCC who underwent TACE showed similar survival and safety profile irrespective of age[64,65]. In a large retrospective study from Korea, the authors found that TACE was associated with even better results in elderly than in younger patients, with respect to median OS and disease-specific survival (15.2 mo *vs* 8.7 mo, $P < 0.001$) without significant differences in terms of TACE-related mortality[43]. In an "in field" study from Italy, the authors reported similar outcomes in elderly and younger patients treated with TACE[66]. These data suggest that in the elderly population, TACE should be part of therapeutic armamentarium for HCC being effective with a satisfactorily safety profile.

SORAFENIB

Sorafenib (Nexavar, Bayer Healthcare Pharmaceuticals-OnyxPharmaceuticals), is a multikinase inhibitor which exerts a proven anti-angiogenetic and anti-proliferative activity on several solid tumors. Since 2008, it has been approved for treatment of HCC thanks to the results of two international randomized controlled trials[67,68], which were however conducted in well-selected patients with a mean age of about 60 years and without relevant comorbidities.

The efficacy of sorafenib in elderly patients is supported by a number of studies[69-72], which showed similar, or even a trend to longer, overall survival and time to progression in elderly patients compared with younger subjects. Overall, evidence collected to date shows that the efficacy and safety profile of sorafenib is not influenced by age[73,74]. A more strict monitoring might be considered in the elderly because of increased risk in developing comorbidities[73].

CONCLUSION

Since the progressive ageing of the population, the number of elderly HCC patients will increase in the next future. Unfortunately, international guidelines do not specifically address this aspect which, on the other hand, is relevant in clinical practice. Importantly, elderly patients often carry comorbidities and, in clinical practice, comorbidities contribute to poor adherence to guidelines recommendations.

Available data seem to indicate that, beside OLT, any other therapeutic option according to HCC stage, liver function and general clinical conditions, should be offered to aged patients since the expected efficacy depend on HCC stage rather than on the actual age of the patient. Thus a nihilistic attitude of physicians towards under- or no-treatment should be discouraged. It is important to note however, that these recommendations are based on data mostly obtained in carefully selected patients. The decision whether or not to start treatment should therefore follow a patient-by patient strategy, discussed within a multidisciplinary team and shared with patient and relatives taking in great consideration the balance between clinical benefit, risks and life expectancy.

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REVIEW

MicroRNAs dysregulation in hepatocellular carcinoma: Insights in genomic medicine

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Abstract

Hepatocellular carcinoma (HCC) is the leading primary liver cancer and its clinical outcome is still poor. MicroRNAs (miRNAs) have demonstrated an interesting potential to regulate gene expression at post-transcriptional level. Current findings suggest that miRNAs deregulation in cancer is caused by genetic and/or epigenetic, transcriptional and post-transcriptional modifications resulting in abnormal expression and hallmarks of malignant transformation: aberrant cell growth, cell death, differentiation, angiogenesis, invasion and metástasis. The important role of miRNAs in the development and progression of HCC has increased the efforts to understand and develop mechanisms of control overt this single-stranded RNAs. Several studies have analyzed tumoral response to the regulation and control of deregulated miRNAs with good results *in vitro* and *in vivo*, proving that targeting aberrant expression of miRNAs is a powerful anticancer therapeutic. Identification of up and/or down regulated miRNAs related to HCC has led to the discovery of new potential application for detection of their presence in the affected organism. MiRNAs represent a relevant new target for diagnosis, prognosis and treatment in a wide variety of pathologic entities, including HCC. This manuscript intends to summarize current knowledge regarding miRNAs and their role in HCC development.

Key words: Hepatocellular carcinoma; MicroRNAs; Regulation; Therapeutic targets

Core tip: MicroRNAs are implicated in the control of gene expression which enable them a relevant new target for diagnosis, prognosis and treatment in a wide variety of pathologic entities, including hepatocellular carcinoma (HCC). This manuscript represents an attempt to summarize current knowledge regarding miRNAs and

their role in HCC development.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the leading primary liver cancer and represents the fifth most common cause of cancer in men, the seventh in women, and is considered the third most frequent cause of cancer-related death worldwide[1]. Almost 85% of new cases occur in developing countries, with highest incidence in areas located in sub-Saharan Africa, east and southeast Asia but also Melanesia and Micronesia/Polynesia; whereas low-incidence areas include northern and Western Europe and North America[1,2]. Nonetheless, clinical outcome of HCC is still poor, which can be attributed to lack of reliable markers for early diagnosis, resistance to treatment, tumor recurrence, and metastasis. Recent evidence suggests a rising incidence of HCC-related deaths in the United States, and during the last two decades, the incidence of HCC in this country has tripled with no difference in 5-year survival rate (12%)[3,4].

HCC develops within an established background of chronic liver disease like cirrhosis due to hepatitis B virus (HBV) and/or HCV, non-alcoholic steatohepatitis, autoimmune hepatitis, iron overload syndromes, diabetes, alcohol abuse, smoking, oral contraceptive use and aflatoxin exposure[5-8].

HCC is believed to be a multistep process, though despite an increasing knowledge of molecular mechanisms inducing hepatocarcinogenesis, poor prognosis of HCC patients reflects the failure to block and reverse the steps of molecular transformation[9,10].

Up to now, alpha-fetoprotein (AFP) along with ultrasounds every 6-12 mo remains as the most commonly used approach to monitoring patients at high risk for HCC[6,11]. Unfortunately the use of both diagnostic tools not only fails to increase detection rates, but also raises false positive uncertainties[12].

Recent studies have demonstrated evidence that anomalous expression of specific miRNAs are implicated in a broad spectrum of human ailments, including rheumatic diseases[13-15], diabetes/insulin resistance[16-18], cardiovascular disease[19-21], renal disease[22] and a wide variety of cancers[23].

Last but not least, the aim of this review is to provide an update in the field of miRNAs and their application in different aspects of HCC.

MIRNAS OVERVIEW AND ITS ROLE IN CANCER DEVELOPMENT

MicroRNAs (miRNAs) are defined as non-coding single-stranded RNAs (ssRNAs) of 19-25 nucleotides in length that are generated from endogenous hairpin-shaped transcripts[24]. MiRNAs were first reported by Lee *et al*[25], who described a small noncoding RNA encoded by the lin-4 locus associated to the developmental timing of the nematode *Caenorhabditis elegans*. Since that moment, thousands of miRNAs have been identified in a wide variety of organisms, including mammals and specifically humans. Actually, we know that about 3% of human genes encode miRNAs and more than 1500 miRNA genes have been predicted or experimentally shown to play critical roles in normal cellular functions[26-28].

Up to date, miRNAs have demonstrated an interesting potential to regulate gene expression at post-transcriptional level, binding through partial complementarity to target mRNAs, and mainly leading to mRNA degradation or translation inhibition[29]. Imperfect base pairing between miRNAs and mRNAs is common and enables miRNAs to regulate a broad, but specific set of genes[30].

The first evidence of the involvement of miRNAs in human cancer was reported in chronic lymphocytic leukemia (CLL) patients in 2002, when Calin *et al*[31] showed miR-16-1 and miR-15a deletion in chromosome 13q14 in more than 59% of CLL patients. Recently, miRNAs alterations have been described in different types of cancer, including CLL, acute promyelocytic leukemia, acute myeloid leukemia, multiple myeloma, monoclonal gammopathy of undetermined significance, non-Hodgkin lymphoma, breast cancer, esophageal cancer, gastric cancer, clear-cell kidney cancer, cervical cancer, and others[23].

Current findings suggest that miRNAs deregulation in cancer is caused by genetic and/or epigenetic, transcriptional, and post-transcriptional modifications resulting in abnormal expression and hallmarks of malignant transformation: aberrant cell growth, cell death, differentiation, angiogenesis, invasion and metastasis[32,33]. This knowledge has established miRNAs as potential diagnostic biomarkers or even as new therapeutic targets in the fight against cancer.

The difficulty of miRNA target prediction and biological validation has been a major obstacle to miRNA research. Experimental identification of miRNAs is difficult to isolate by cloning due to low expression, low stability, tissue specificity and problems in cloning procedures[34].

MIRNAS IN HCC

As discussed before, miRNAs have important functions in cancer development because of their relevant role in regulation of cell proliferation, avoidance of apoptosis

(cell perpetuation) and metastasis.

Recently, the identification of up and/or down regulated miRNAs related to HCC has led to the discovery of new potential application for detection of their presence in the affected organism. Up to now, every week appears new evidence of miRNAs with potential effect on carcinogenesis; therefore, in this review we expose the most relevant findings on the field of miRNAs in HCC. To provide an easy comprehension of the data, we have classified our findings based in the up or down regulation status of the most relevant minas implicated in HCC development[35].

Up-regulated miRNAs in HCC

The role of several miRNAs has been studied in other malignances, this is the case of miR-181a which is associated with malignancies such as chronic lymphocytic leukemia and acute myelogenous leukemia[36], and has been linked to improved survival and decrease recurrence in gliomas, where it seems to be an inhibitor of oncogenesis and tumor growth with importance in the development of epithelial cell adhesion molecule$^+$/AFP$^+$ HCC associated with increased metastases and poor survival. Bhattacharya *et al*[37] analyzed the role of osteopontin (OPN) in HCC, and their findings suggested that OPN confer a prometastatic phenotype to cancer cell lines. Recent findings have described that miR-181 are up-regulated in hepatic stem cell populations and HCC cells with progenitor cell features, implying that miR-181 functions in maintaining an undifferentiated state of hepatic progenitor cells. In this regard, evidence suggests that miR-181 may activate hepatic progenitor cells and HCCs through two cellular signaling pathways: (1) blockage of HCC cell differentiation through inhibition of GATA6 or CDX2, two transcriptional activators regulating hepatocyte differentiation; and (2) activation of Wnt/β-catenin pathway by down-regulating NLK, a Wnt/β-catenin signaling inhibitor[38].

MiR-21 overexpression is found in HCC cells and has been linked to inhibition of apoptosis and promotion of cell proliferation. Connolly *et al*[39], studied the role of miR-21 in cell invasion and migration, and found that overexpression of this miRNA increases matrix metalloproteinase-9 (MMP-9) activity in multiple cell lines. These findings described the role of MMP-9 expression with invasive and/or metastasic phenotypes of tumors. Other mechanism of metastases identified the role of tumor suppressor RECK, in conjunction with RHOB, in regulating the *in vitro* metastatic properties, being associated with poor prognosis[39].

MiR-151 is localized within intron 22 of focal adhesion kinase (FAK), which is often overexpressed in human tumors and promotes cancer cell invasion and metastasis. A study carried-out by Ding *et al*[40] found that suppression of p53 can increase the expression of both FAK and miR-151 simultaneously, suggesting that p53 may be a potential transcriptional regulator for FAK and miR-151 in liver cancer cells. Other description made by this team revealed that *RhoGDIA* is a direct and functional target for miR-151, which once suppresses *RhoGDIA* expression activate Rac1, Cdc42 and Rho GTPases, and this inhibitory effect may work synergistically with FAK signaling to promote cell motility and invasion. This situation indicates that it may be a general mechanism for the metastasis of human cancer cells.

Upregulation of miR-191 after hepatocyte injury has been linked with extensive changes in gene expression. The most affected pathways are transforming growth factor beta (TGF-β) and mitogen-activated protein kinases (MAPK) which play a significant role in hepatocarcinogenesis. TGF-β pathway regulates cell proliferation, differentiation, and adhesion. While MEPK signaling pathway is also involved in diverse cellular processes such as cell survival, differentiation, and proliferation[41].

Overexpression of miR-221 is present in almost 71% of HCC and plays an important role in HCC development due to its ability to modulate the expression of the oncogenic proteins c-kit and cyclin-dependent kinase inhibitors CDKN1B/p27 and CDKN1C/p57, promoting cancer cell proliferation. Dysregulation of CDKN1B/p27 exhibits a relevant prognostic significance, being associated with advanced tumor staged, poor survival and recurrence of small HCC. Whereas CDKN1C/p57, has been linked with higher biological aggressiveness, advanced stage, poor differentiation, larger size, portal invasion and high proliferative activity[42]. Other studies showed that miR-221 dysregulation alters G1/S transition inhibitors, where p27 and p21 proteins are frequently down-regulated in HCC, while TGF-β proteins were frequently up-regulated. These alterations lead in loss of control of the transition G1/S in HCC cells, which result in cellular proliferation and metastasis improvement[43]. Furthermore, new evidence suggests a wider role of miRNA in HCC[44], and recently Gramantieri *et al*[45], described how throughout a pro-apoptotic molecule called Bmf, miR-221 can simultaneously affect proliferation and apoptosis. Bmf is involved in the balance of pro-apoptotic and anti-apoptotic stimuli in Bcl-2/Bcl-xL-induced apoptosis and also seems to follow TGF-β up-regulation[45].

MiR-224 over-expression found in HCC tissues suggests its key role in the malignant phenotype of hepatocarcinoma cells. Recent findings affirmed that miR-224 can modulate cell proliferation and has an important role in cell migration and invasion. Alteration of molecules PAK4 and MMP-9 are considered as the misbalance responsible of the carcinogenic role of miR-224[46].

MiR-183 in the liver acts as negative regulator of programmed cell death 4 (PDCD4) molecule acting at posttranscriptional level which has been found to inhibit activator protein-1 (AP-1) mediated trans-activation and to induce expression of the cyclin-dependent kinase inhibitor p21. MiR-183 up-regulation and subsequent loss of PDCD4 improves cell growing and thereby facilitates cancer development[47]. PDCD4 down-regulation was

Table 1 Upregulated miRNAs in hepatocellular carcinoma

MiRNA	Cellular process	Ref.
MiR-10a	Epithelial to mesenchymal transition and metastasis	[50]
MiR-130a	Drug resistance	[51]
MiR-135a	Metastasis	[52]
MiR-143	Metastasis	[53]
MiR-155	Proliferation and tumorigenesis	[54]
MiR-18a	Proliferation	[55]
MiR-181b	Cell growth, tumorigenesis and metastasis	[56]
MiR-182	Metastasis	[57]
MiR-183	Apoptosis	[47]
MiR-21	Metastasis and drug resistance	[39,58,59]
MiR-210	Metastasis, apoptosis and proliferation	[60,61]
MiR-216a	Tumorigenesis	[62]
MiR-221	Apoptosis, proliferation and angiogenesis	[42,45,63,64]
MiR-224	Metastasis, proliferation and apoptosis	[65-67]
MiR-23a	Gluconeogenesis	[68]
MiR-373	Cell cycle	[69]
MiR-301a	Metastasis	[70]
MiR-490-3p	Epithelial to mesenchymal transition	[71]
MiR-519d	Proliferation, invasion and apoptosis	[72]
MiR-550a	Metastasis	[73]
MiR-590-5p	Metastasis and proliferation	[74]
MiR-615-5p	Cell growth and migration	[75]
MiR-657	Proliferation	[76]
MiR-96	Proliferation	[77,78]
MiR-222	Metastasis	[79]

MiRNAs: MicroRNAs.

previously recognized in human colorectal cancer and melanoma[48,49].

Other up-regulated miRNAs related to hepatocarcinogenesis are included in Table 1.

Down-regulated miRNAs in HCC

MiR-122 is highly abundant in liver, accounting for 70% of total liver miRNA reported[80-82]. Previous reports had shown its positive regulation of lipid metabolism and disease, but recent knowledge has established an important role of miR-122 in hepatocarcinoma/hepatoma, acting as tumor suppressor gene frequently down-regulated in HCC cell lines and correlated with clinical parameters as etiology, tumor size and differentiation grade. Recent findings suggest that miR-122 inhibits and controls all characteristic properties of cancer cells such as cell cycle, clonogenic survival, anchorage-independent growth, migration, invasion, epithelial-mesenchymal transition and mutagenesis[83-85]. The mechanisms of this dysregulation are unknown, but studies have provided genes and molecules implicated which include ADAM10, Igf1R, SRF, peroxiredoxin 2, members of the septin family like SEPT2 and SEPT9, vimentin, MMP-7, Aldoase A, the muscle isoform of pyruvate kinase (PKM2), and cyclin G1[83-87]. Coulouarn *et al*[88] showed that repression of miR-122 was characteristic of HCC displaying either a hepatoblast, c-Met or late TGF-β signature; these results showed that HCC cell lines exhibit a more invasive phenotype once decreased miR-122 expression is present. Other study correlated high AFP level with more aggressive properties of HCC. These findings correlated also with lower rates of recurrence-free survival and lower overall survival due to increased expression of CUX1, a direct target of miR-122[89].

Decreased levels of miR-26 in HCC have been associated with poor prognosis and are considered predictive of therapeutic response to interferon-α. Recent studies have reported that animals treated systemically with miR26 presented tumor regression. Recent studies elucidated the role of miR-26 in hepatocyte proliferation confirming that E2 promotes liver cancer cells growth *via* the E2-ERα pathway and suggested that miR-26 significantly down-regulates ERα preventing hepatoma cell growth, suggesting anti-carcinogenic activities in women[90,91]. Also, miR-26 directly or indirectly regulates expression of a wide variety of genes by down-regulating AFP, PCNA, PR, CEA, nuclear factor-κB and interleukin-6 or increasing P53 and PTEN[90-92].

MiR-34a has been considered a direct transcriptional target of p53 and is commonly reduced or deleted in HCC and other cancers[93]. To date, there are more than 34 proteins altered by miR-34a down-regulation, which include LMNA, ALDH2, MACF1, LOC100129335, GFAP and c-Met as targets of miR-34a with a crucial role in hepatocarcinogenesis[94]. Likewise, down-regulation of miR-34 has shown to down-regulate CyclinD1-CDK6 complex, which is one of the critical positive regulators during G1/S phase transition and a major checkpoint for cell progression. These alterations proved that miR-34a deregulation has the capacity to increase adhesion of tumoral cells to regional lymph nodes improving metastasis[95,96].

Recently, it has been demonstrated that miR-29b is capable of repressing tumor angiogenesis, invasion and metastasis in normal subjects by suppressing MMP-2. Data provided by Fang *et al*[97], suggest that miR-29b deregulation result in enhanced MMP-2 level in the tumor microenvironment, which in turn activates vascular endothelial growth factor receptor-2 (VEGFR-2) in endothelial cells promoting angiogenesis. Conclusions provided by Fang *et al*[97], showed inhibitory effects on invasion and metastasis and established MMP-2 as a relevant protein implicated in tumoral growth and metastasis.

MiR-145 forms a double negative feedback loop with key stemness factors OCT4, SOX2, and KLF. And, at the same time, OCT4 binds to the miR-145 promoter and suppresses its expression. Down-regulation of miR-145 in human embryonic stem cells impairs its differentiation and enhances stem cell self-renewal, these findings suggest an important role of miR-145 in carcinogenesis[98]. A study published by Gao *et al*[99], studied the role of miR-145 in hepatocarcinogenesis and they concluded that down-regulation of miR-145 favors cellular proliferation and migration, suggesting that miR-145 acts as a negative regulator of HCC development.

The analysis of miR-199 down-regulation showed new specific targets like CD44, a member of trans-

Table 2 Downregulated microRNAs in hepatocellular carcinoma

MiRNA	Cellular process	Ref.
Let-7a	Apoptosis and proliferation	[107,108]
Let-7b	Apoptosis and proliferation	[109]
Let-7c	Apoptosis, proliferation and cell growth	[110-112]
Let-7d	Apoptosis and proliferation	[107]
Let-7f-1	Apoptosis and proliferation	[107]
Let-7g	Apoptosis and metastasis	[113-115]
MiR-1	Proliferation	[116]
MiR-34a	Metastasis	[96]
MiR-101	Apoptosis and DNA methylation	[110,117,118]
MiR-122	Apoptosis, metastasis and angiogenesis	[119-122]
MiR-124	Proliferation	[123]
MiR-125a	Proliferation, metastasis and metabolism	[124-126]
MiR-125b	Proliferation, metastasis, angiogenesis, apoptosis and histone modification	[110,125-127]
MiR-139	Metastasis	[128,129]
MiR-138	Cell cycle	[130]
MiR-145	Cell growth and tumorigenesis	[131]
MiR-195	Tumorigenesis, cell cycle and apoptosis	[132,133]
MiR-199a-3p	Drug resistance and cell growth	[101,134]
MiR-199a-5p	Invasion and autophagy	[135]
MiR-200a	Proliferation and metastasis	[136]
MiR-203	Proliferation	[137]
MiR-214	Cell growth, metastasis and angiogenesis	[138,139]
MiR-219-5p	Proliferation	[140]
MiR-223	Proliferation	[141]
MiR-26a/b	Cell cycle	[142]
MiR-29a	Proliferation	[143]
MiR-34a	Metastasis	[96,144]
MiR-375	Autophagy	[145]
MiR-376a	Apoptosis and proliferation	[146]
MiR-449	Proliferation and apoptosis	[147]
MiR-450a	Proliferation	[148]
MiR-520b	Cell growth and proliferation	[149]
MiR-7	Tumorigenesis and metastasis	[150]

MiRNAs: MicroRNAs.

membrane glycoproteins which acts mainly as receptor of hyaluronic acid, being involved in cell-cell interactions, cell adhesion and migration. Studies have demonstrated that inhibition of CD44 enhances apoptosis and improves chemosensitivity, diminishes tumorigenesis and invasion[100]. Interestingly, miR-199 also plays a relevant role in regulation of mammalian target of rapamycin (mTOR) which stands a key role in cell growth, protein translation, metabolism, cell invasion and apoptosis; and c-Met, a proto-oncogene involved in a biological "invasive growth" that result from stimulation of cell motility, invasion, and protection from apoptosis[101,102].

Up-regulation of MKi67 is considered an important risk factor for pathologies in breast, prostate and others cancers like meningiomas, but Hou *et al*[103] found that higher levels in human HCC cells contribute to malignant phenotype. This study recently published showed that in normal situations, miR-519 suppresses cellular growth by MKi67 due to direct binding of the miRNA to an identified target site in the MKi67 3'-UTR where mutation of this region abolishes this effect.

MiR-152 down-regulation was described as a cause of aberrant DNA methylation by targeting DNMT1, and is inversely correlated with DNMT1 expression in HCC. DNMT1 is necessary and sufficient for maintaining global methylation and aberrant CpG island methylation in human cancer cells contributing to pathogenesis of HCC[104].

Recently, an inverse correlation between miR-338 and smoothened (SMO) expression has been elucidated by Huang *et al*[105], where miR-338 showed an important role in suppressing HCC metastasis through down-regulating SMO. MMP-9 expression is increased in HCC, correlates with metastasis and advanced tumor stages, and this study has demonstrated that SMO siRNA can abolish MMP-9 expression. These results indicate that miR-338 suppresses the invasiveness of liver cancer through down-regulation of SMO-induced MMP-9 expression[105].

MiR-101 has been shown to be down-regulated in different tumors like breast, lung and pituitary adenoma, but Li *et al*[106] have demonstrated that its under-expression also has an important role in cell invasion and migration in HCC. This oncogenic activity is attributed to FBJ murine osteosarcoma (FOS), which in normal tissues is negatively regulated by miR-101 at posttranscriptional level *via* a specific target site within the 3'-UTR. Down-regulation of miR-101 may contribute to the high expression level of FOS protein, which activates the AP-1 family of transcription factors (c-fos and c-jun). Both, c-fos and c-jun can induce epithelial-mesenchymal transition, a hallmark of metastasis and invasive growth associated with loss of cell polarity in epithelial cells. Therefore, according with Li *et al*[106] regulation of miR-101 could be a potentially suitable candidate for anticancer therapy.

Additional down-regulated miRNAs are included in Table 2.

DISCUSSION

Thus far, more than 800 human miRNAs have been described and speculations about the total number of human miRNAs have exceeded 1000[106]. In human cancer, every type of tumor shows a miRNA profile significantly different compared with normal cells from the same tissue.

Single nucleotide polymorphisms in miRs and their targets have been associated with risk of various cancers because changes in the expression pattern of a gene could therefore influence a person's risk of illness. Noteworthy, miRs are considered promising prognostic markers of HCC. Some studies have shown that miRs are protected from enzymatic cleavage by RNAses in blood, and therefore their expression profile in serum or plasma could also be utilized as novel diagnostic and prognostic markers[151,152].

Taking into account all this great deal of data, miRNAs issue is one of the most complex topics in oncology due to its wide range of actions as either oncogenes or tumor-suppressors genes in HCC. These facts have led investigators to device two approaches for developing miRNA-based therapies: antagonists and/or mimics[30].

The important role of miRNAs as players in the development and progression of HCC has increased the efforts to understand and develop mechanisms of control overt this ssRNAs. In the last years, several studies have been designed to analyze tumoral response to the regulation and control of deregulated miRNAs with good results *in vitro* and *in vivo,* proving that targeting aberrant expression of miRNAs is a powerful anticancer therapeutic[9]. Recent data showed that tumor suppressive miRs expressed in normal liver are down-regulated in tumor tissues during tumorigenesis and metastasis. Hence, a potentially plausible strategy would be to replenish those miRs systemically in HCC patients (miR-181, miR-29, miR-221, miR-122, miR-29, miR-199, *etc*.) to restore altered pathways balance, and stimulate and/or increase cellular mechanisms to regulate cell proliferation, cell cycle regulation, cell migration and invasion and apoptosis[35].

One of the biggest challenges to translate this knowledge to humans resides that every miRNA may target several mRNAs[78]. This situation empowers selective delivery a crucial issue, which calls for alternate targeted delivery strategy more refined and accurate. The use of viral vectors represents a promising approach[5].

CONCLUSION

MiRNAs are implicated in the control of gene expression which enable them a relevant new target for diagnosis, prognosis and treatment in a wide variety of pathologic entities, including HCC. This manuscript represents an attempt to summarize current knowledge regarding miRNAs and their role in HCC development.

We believe that miRNA is one of the most promising and challenging opportunities to classify and attack cancer. However, translation of knowledge from experimental models to humans remains as a critical point due to the wide and different range of effects caused by each miRNA from cell to cell. Thus, cell-specific delivery most be improved to increase tumoral-specificity and then be considered as a potential therapy in human cancer.

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REVIEW

Current and future directions for treating hepatitis B virus infection

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Abstract

Hepatitis B virus (HBV) persistently infects approximately 350 million people, and approximately 600000 liver-related deaths are observed per year worldwide. HBV infection is also one of the major risk factors for hepatocellular carcinoma (HCC). The persistence of serum hepatitis B e antigen (HBeAg) and high level of serum HBV DNA are thought to reflect a high HBV replication status in hepatocytes, causing cirrhosis, HCC and liver-related deaths. It has been reported that antiviral therapy, such as peginterferon and nucleos(t)ide analogues (NUCs), could suppress liver-related death by inhibiting the HBV DNA levels and inducing seroconversion from HBeAg to antibody to HBe antigen. Currently, peginterferon is widely used, but there are also several disadvantages in the use of peginterferon, such as various adverse events, the administration route and duration. It is difficult to predict the effects of treatment and interferon is contraindicated for the patients with advanced fibrosis of the liver and cirrhosis. With respect to NUCs, entecavir and tenofovir disoproxil fumarate are current the first-choice drugs. NUCs can be administered orally, and their anti-viral effects are stronger than that of peginterferon. However, because cessation of NUC administration leads to high levels of viral replication and causes severe hepatitis, they must be administered for a long time. On the other hand, the use of both interferon and NUCs cannot eliminate covalently closed circular DNA of HBV. In this review, we evaluate the natural course of chronic HBV infection and then provide an outline of these representative drugs, such as peginterferon, entecavir and tenofovir disoproxil fumarate.

Key words: Hepatocellular carcinoma; Peginterferon; Nucleotide analogue; Chronic hepatitis B

Core tip: Chronic hepatitis B virus (HBV) infection is one of the major causes of hepatocellular carcinoma, which is a cancer with poor prognosis. We reviewed the natural

course of HBV infection and current standard therapies for chronic HBV infection. Peginterferon and nucleos(t)ide analogues, such as entecavir and tenofovir disoproxil fumarate, have several drug-specific advantages and disadvantages. It is difficult to eliminate covalently closed circular DNA of HBV with these current standard therapies. Further improvements of the therapeutic options for HBV infections should be needed.

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INTRODUCTION

Approximately 350 million people are persistently infected with hepatitis B virus (HBV) and there are 600000 HBV-related deaths annually worldwide[1]. It has been reported that more than 90% of patients infected with HBV in their infancy or childhood become chronic HBV carriers[1]. Of them, approximately 15%-40% develop chronic hepatitis B. In the patients with chronic hepatitis B, approximately 90% could achieve seroconversion of hepatitis B e antigen (HBeAg) to antibody to HBe antigen (anti-HBe) and become inactive carriers. However, approximately 10% of patients with chronic hepatitis B have chronic active hepatitis and develop cirrhosis at a rate of approximately 2% per year, leading to liver failure and/or hepatocellular carcinoma (HCC)[2-5]. Globally, HBV infection is one of the major risk factors of HCC, and it accounts for up to 50% of all HCC patients. Positive serum HBeAg and a high level of serum HBV DNA are indicative of high HBV replication in the liver[6-8]. Therefore, it is important to suppress HBV replication to prevent hepatic failure and the development of cirrhosis and HCC. To prevent the disease progression, peginterferon and nucleos(t)ide analogues (NUCs) are now available as antivirals against HBV[9-12].

In general, the natural history of chronic HBV infection in birth or early childhood is divided into five phases as follows (Figure 1). In phase 1 (immune tolerance phase/asymptomatic carrier phase), HBV is actively replicating, but the host lacks an immune response. The serum alanine aminotransferase (ALT) level is within the normal limit and liver inflammation is almost absent. In phase 2 (immune clearance phase), in adulthood, the immune response to HBV becomes active, and an elevated serum ALT level and active hepatitis are observed. In phase 3 (inactive phase), as a result of an immune response, HBeAg is lost, anti-HBe emerges, the serum HBV DNA level is suppressed and liver inflammation is low[13]. Some patients cannot achieve seroconversion from HBeAg to anti-HBe and HBeAg persists as positive. For most of those with positive HBeAg, active hepatitis persists, and they often rapidly proceed to cirrhosis (HBeAg-positive hepatitis). In approximately 10%-20% of HBeAg-negative carriers, HBV DNA replication is reactivated and active hepatitis flares again (HBeAg-negative hepatitis) (phase 4)[14]. It should be noted that there are some developments in HCC even at low rates in this phase[15]. For approximately 4% to 20% of HBeAg-negative carriers, anti-HBe is lost and HBeAg reappears again (reverse seroconversion). In the natural course of HBeAg-negative carriers, HBs antigen (HBsAg) converts to negative and antibody to HBs antigen (anti-HBs) develops at a rate of 1% per year (phase 5, remission phase). In this phase, the both blood test and liver histology findings might be improved.

For acute-on-chronic liver failure and HCC, the risk factors are obvious and antiviral therapies could reduce the risk of developing acute-on-chronic liver failure and HCC[16]. In general, the indication and selection of antiviral therapies for persistent HBV infection is decided according to the age, disease phase, fibrosis stage and inflammatory activity of the liver, and risk of disease progression. In the immune tolerance phase, the rate of HBV clearance from the hepatocytes is very low because of the lack of a host immune response. In the low replication phase (inactive carriers), antiviral therapy may not be indicated if the liver histological findings are mild and the serum ALT level is within normal limits. In the remission phase (negative HBsAg), if HBV DNA is not detected, antiviral therapy may not be indicated because hepatitis calms down and the HCC development rates decrease[17] while NUC administration may be stopped. In young patients with HBeAg-positive chronic hepatitis and elevated serum ALT levels, there is a 7%-16% possibility of HBeAg-negative conversion. Then, strict observation without treatment may be chosen if there is no advanced fibrosis or possibility of fulminant hepatitis[16].

HBV-INFECTED PATIENTS WITH CIRRHOSIS

While cirrhotic patients are thought to be therapeutic indication even if HBeAg is negative, the serum ALT level is normal and serum HBV DNA level is suppressed at a low level. If advanced fibrosis is clinically suspected, the assessment of fibrosis should be performed by liver biopsy, abdominal ultrasonography, elastography or abdominal computed tomography[18-22]. If advanced fibrosis is observed, antiviral therapy should be started. HBV carriers who are not asymptomatic or inactive carriers are indicated to receive antiviral therapy, and inactive carriers with advanced fibrosis and a high serum HBV DNA level are also indicated to receive antiviral therapy.

At present, the complete elimination of covalently closed circular DNA (cccDNA) in nucleus of liver cells[23] seems difficult using peginterferon and NUCs. The best surrogate markers for antiviral treatment against HBV

Table 1 Treatment efficacy at 24 wk after the end of peginterferon treatment in hepatitis B e antigen-positive chronic hepatitis B

Ref.	No. of patients	Formula of therapy	Seroconversion from HBeAg to anti-HBe (%)	Suppression of HBV DNA (%)	Normalization of ALT (%)	HBsAg loss (*n*)
Cooksley *et al*[27]	51	IFNα-2a 4.5 MIU three times weekly for 24 wk	25	25[a]	26	0
	49	Peg-IFNα-2a 90 μg weekly for 24 wk	37	43[a]	43	0
	46	Peg-IFNα-2a 180 μg weekly for 24 wk	35	39[a]	35	0
	48	Peg-IFNα-2a 270 μg weekly for 24 wk	27	27[a]	31	0
Lau *et al*[28]	214	Peg-IFNα-2a 180 μg weekly plus placebo for 48 wk	32	32[b]	41	8
	271	Peg-IFNα-2a 180 μg weekly plus LAM 100 mg daily for 48 wk	27	34[b]	39	8
	272	LAM 100 mg/d for 48 wk	19	22[b]	28	0
Chan *et al*[29]	50	Peg-IFNα-2b 1.5 μg/kg weekly for 32 wk plus LAM 100 mg daily for 52 wk	36	36[a]	50	1
	50	LAM 100 mg daily for 52 wk	14	14[a]	30	0
Liaw *et al*[30]	140	Peg-IFNα-2a 90 μg weekly for 24 wk	14	21[c]	30	1
	136	Peg-IFN α-2a 180 μg weekly for 24 wk	22	21[c]	30	0
	136	Peg-IFNα-2a 90 μg weekly for 48 wk	25	32[c]	43	3
	136	Peg-IFNα-2a 180 μg weekly for 48 wk	36	42[c]	52	3

a < 500000 copies/mL; b < 100000 copies/mL; c < 20000 copies/mL. LAM: Lamivudine; Peg-IFN: Peginterferon; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; Anti-HBe: Antibody to HBe antigen; ALT: Alanine aminotransferase.

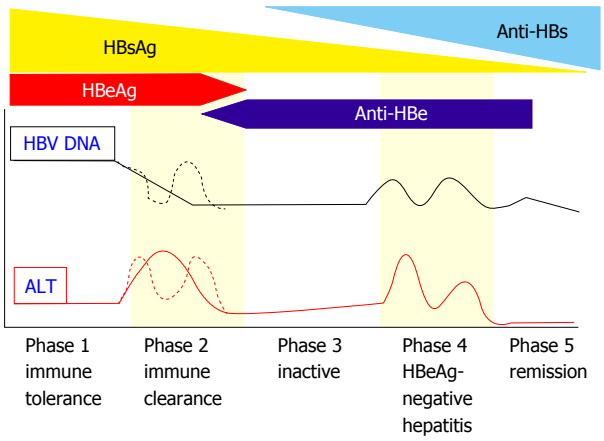

Figure 1 Natural course of hepatitis B virus infection[116]. HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B s antigen; Anti-HBe: Antibody to HBe antigen; Anti-HBs: Antibody to HBs antigen.

are HBsAg as a long-term marker as well as sustained normalization of the serum ALT level, negative serum HBV DNA level and negative HBeAg as short-term markers[16].

PEGINTERFERON THERAPY

Greenberg *et al*[24] reported the usefulness of interferon therapy for chronic hepatitis B in 1976. Interferon exerts antiviral activity, cell growth inhibition and immunomodulatory effects. It binds to the interferon receptors of hepatocytes, activates tyrosine type protein kinase Janus kinase 1 and induces phosphorylation and dimerization of signal transducer and activator of transcription 1 (STAT1). The STAT1 dimer translocates into the nucleus, induces interferon stimulated genes, and expresses various antiviral proteins that have antiviral effects[25]. The HBeAg-negative conversion rate of the interferon treated group was significantly higher than that of the untreated control group[26]. Interferon is non-antigen specific immunomodulator. Compared with NUCs, one of the advantages of interferon is that its treatment duration is limited and its effect is durable. The other benefit of interferon is that there is no risk of resistance mutants. However, interferon has no direct inhibitory effect on viral replication and its short-term effect, such as suppressing serum HBV DNA level, is inferior to NUCs. The other disadvantage of interferon is the difficulty in predicting the treatment effect and several adverse events, such as flu-like syndrome. Additionally, it is difficult to use interferon on patients with advanced liver fibrosis and cirrhosis.

Compared to standard interferon, peginterferon-alpha has a long half-life and gains its long-acting effect through the addition of polyethylene glycol high molecular proteins to interferon. Its administration is performed only once per week. There have been several reports about peginterferon for HBeAg-positive or HBeAg-negative patients (Tables 1-3)[27-35]. In the comparison trial with standard interferon-alpha and peginterferon-alpha in Asia, the combined responses, defined as HBeAg loss, HBV DNA suppression (< 500000 copies/mL) and ALT normalization, were 28% *vs* 12%, respectively (P = 0.036), and the superiority of peginterferon-alpha to standard interferon has been demonstrated[27]. A report comparing three groups of 48 wk of peginterferon-alpha-2a alone, 48 wk of peginterferon-alpha2a plus lamivudine, and 48 wk of lamivudine alone in 814 HBe positive patients reported that the HBeAg seroconversion rates 24 wk after the end of administration were 32%, 27% and 19%, respectively, and the peginterferon-alpha-2a alone group had a significantly higher effect than lamivudine alone group[28]. The HBsAg-negative

Table 2 Long-term treatment efficacy of peginterferon treatment in hepatitis B e antigen-positive chronic hepatitis B

Ref.	No. of patients	Formula of therapy	Seroconversion from HBeAg to anti-HBe (%)	Suppression of HBV DNA (%)	Normalization of ALT (%)	HBsAg loss (*n*)
[c]Buster *et al*[31]	91	Peg-IFNα-2b for 52 wk	35	25[a]	30	7 (8%)
	81	Peg-IFNα-2b plus LAM for 52 wk	25	31[a]	30	12 (15%)
[d]Wong *et al*[32]	85	Peg-IFNα-2b for 32 wk plus LAM for 52 or 104 wk	60	13[b]	57	2 (2.4%)

a < 10000 copies/mL; b < 100 copies/mL. The treatment efficacies were assessed at approximately 3-year follow-up[c] or approximately 5-year follow-up[d]. LAM: Lamivudine; Peg-IFN: Peginterferon; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase.

Table 3 Treatment efficacy of peginterferon treatment in hepatitis B e antigen-negative chronic hepatitis B

Ref.	No. of patients	Therapy regimen	HBV DNA suppression (%)	ALT normalization (%)	HBsAg loss (*n*)
[c]Marcellin *et al*[33]	177	Peg-IFNα-2a 180 μg weekly plus placebo for 48 wk	43[a]	59	7
	179	Peg-IFNα-2a 180 μg weekly plus LAM 100 mg daily for 48 wk	44[a]	60	5
	181	LAM 100 mg daily for 48 wk	29[a]	44	0
[c]Papadopoulos *et al*[34]	88	Peg-IFNα-2b 1.5 μg/kg weekly plus LAM 100 mg daily for 48 wk	59 (60 IU/mL below)	27	NA
	35	Peg-IFNα-2b 1.5 μg/kg weekly for 48 wk	42	40	NA
[d]Marcellin *et al*[35]	116	Peg-IFNα-2a 180 μg weekly plus placebo for 48 wk	28[b]	31	9 (8%)
	114	Peg-IFNα-2a 180 μg daily plus LAM 100 mg daily for 48 wk	25[b]	31	9 (8%)
	85	LAM 100 mg daily for 48 wk	15[b]	18	0 (0%)

a < 20000 copies/mL; b < 10000 copies/mL. The treatment efficacies were assessed at 24-wk follow-up[c] or approximately 3-year follow-up[d]. LAM: Lamivudine; Peg-IFN: Peginterferon; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; NA: Not available.

conversion rate was 3%[28]. In a NEPTUNE trial exploring the appropriate dose and duration of interferon treatment, the HBeAg seroconversion rate of 180 μg peginterferon-alpha-2a for 48 wk was significantly higher than that of 24 wk or 90 μg. Therefore, 180 μg peginterferon-alpha-2a administrations for 48 wk were considered the standard treatment[30]. The durable effect after stopping (peg)interferon administration is one specific advantage of this therapy. In another report[31], 81% of HBeAg-positive patients who achieved HBeAg-negative conversion by peginterferon-alpha-2b sustained their effect at years 3 after stopping interferon administration, and 27% of patients who could not achieve HBeAg-negative conversion at week 26 achieved HBeAg-negative conversion at years 3 (Table 2)[31]. In that report[31], 11% of all patients and 30% of patients who achieved HBeAg-negative conversion at month 6 achieved HBsAg-negative conversion even though 31% of all patients in this trial were genotype A and 47% were given additional NUCs. In a multicenter, randomized control trial conducted in Europe on peginterferon-alpha-2a for HBeAg-negative patients comparing three groups treated with 48 wk of peginterferon-alpha-2a alone, 48 wk of peginterferon-alpha-2a plus lamivudine and 48 wk of lamivudine alone, the serum ALT normalization rates 24 wk after end of administration were 59%, 60% and 44% and the serum HBV DNA negative rates were 43%, 44% and 29%, respectively (Table 3)[33]. HBsAg converted to negative in 7 patients in the peginterferon-alpha-2a alone group and 5 patients in the peginterferon-alpha-2a plus lamivudine group. A meta-analysis comparing peginterferon with NUCs has already been published and it was reported that peginterferon-alpha achieved a higher serum HBsAg-negative conversion rate compared to lamivudine monotherapy[36]. In a European multicenter trial conducted over 3 years, 8.7% of all patients and 44% of HBV DNA-negative patients treated with peginterferon-alpha-2a alone had HBsAg-negative conversion[35]. For a longer duration of peginterferon administration in HBeAg-negative patients, 180 μg of peginterferon-alpha-2a administered for 48 wk or 96 wk (49 wk or later, the peginterferon dose was down to 135 μg) were compared and the serum HBV DNA suppression rates (< 2000 IU/mL) were 29% *vs* 12% and serum HBsAg-negative conversion rates were 0% *vs* 6%, respectively. Also, the 96 wk administration was superior to 48 wk administration[37]. Patients in this study were infected with HBV genotype D. The HBeAg-negative patients treated by peginterferon-alpha-2a had worse results than HBeAg-positive patients treated by the same regimen.

While the prediction of the treatment effect by pre-treatment factors is difficult for (peg)interferon therapy, some reports have showed that measuring the serum HBsAg level at weeks 12, 24 and 48 after starting interferon administration contributed to predicting the therapeutic response (HBeAg seroconversion, HBV DNA-negative conversion and HBsAg-negative conversion) for both HBeAg-negative and HBeAg-positive patients[38,39].

Table 4 Treatment efficacy of entecavir in chronic hepatitis B

Ref.	No. of patients	HBeAg	Therapy regimen	HBeAg loss (%)/seroconversion from HBeAg to anti-HBe (%)	Undetectable of HBV DNA (%)	Normalization of ALT (%)	HBsAg loss (*n*)
[1]Chang *et al*[66]	354 NUCs - treatment-naive	Positive	ETV 0.5 mg daily for 48 wk	22/21	67	68	6
	355 NUCs - treatment-naive	Positive	LAM 100 mg daily for 48 wk	20/18	36	60	4
[2]Gish *et al*[67]	243 NUCs - treatment-naive	Positive	ETV 0.5 mg daily for 2 yr	NA/31	80	87	18
	164 NUCs - treatment-naive	Positive	LAM 100 mg daily for 2 yr	NA/26	39	79	10
[1]Lai *et al*[70]	296	Negative	ETV 0.5 mg daily for 48 wk	NA/NA	90	78	1
	287	Negative	LAM 100 mg daily for 48 wk	NA/NA	72	71	1

Treatment efficacies were assessed at 48 wk[1], or 2 years[2]. ETV: Entecavir; NUCs: Nucleos(t)ide analogues; NA: Not available; LAM: Lamivudine; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B surface antigen; Anti-HBe: Antibody to HBe antigen.

With respect to the factors affecting the outcome of interferon therapy, the HBV genotype[40-42], age[43] and fibrosis of the liver[44] were reported to affect the therapeutic outcome of standard interferon. On the other hand, peginterferon is highly effective and the age and HBV genotypes are no longer related to the treatment effect of peginterferon except for HBV genotype A[45,46]. For the other HBV genotypes, the therapeutic effects of genotypes C and B for HBeAg-positive and HBeAg-negative patients have been reported to be equivalent[28,35,47-49]. The pretreatment level of HBsAg could not predict the treatment effect, but its reduction rate and level during treatment can predict the therapeutic effect for both HBeAg-positive[39,50] and HBeAg-negative patients[48,51], and it is thought to be useful marker for predicting the therapeutic effect. Additionally, older age is not reported to be related to the therapeutic effect with current peginterferon[30,46], whereas it has been reported that older age has a favorable effect for HBeAg-positive patients[45,52]. Also, advanced fibrosis of the liver was reported to affect treatment response with current peginterferon for chronic hepatitis B[53]. It was reported that the interleukin-28B (*IL28B*) genotypes affected the HBeAg seroconversion and HBsAg-negative conversion rates[52], although the impact of the *IL28B* gene on the treatment effect of interferon is controversial.

Interferon has immunostimulatory action, and it is generally necessary to consider the acute exacerbation risk of hepatitis by immunological destruction of HBV infected cells, especially for cirrhotic patients. Therefore, interferon therapy is thought to be contraindicated for HBV-related cirrhosis.

NUCS

NUCs specifically inhibit DNA polymerase that HBV DNA itself produces in the reverse transcription process of HBV replication. NUCs strongly inhibit the synthesis of the plus and minus strand chains in the HBV life cycle. The effect is highly specific and efficient. All NUCs can be administered orally and their use is simple. The short-term adverse events of NUCs are rare and mild, and they are effective for either genotype. Furthermore, unlike interferon, they are easy for cirrhotic patients to use. On the other hand, HBV cannot be completely eliminated because NUCs cannot eliminate mRNA or cccDNA in the host nucleus, which acts as a template for HBV DNA.

Once NUC administration is stopped, HBV DNA starts to reappear or increase, and hepatitis recurs in some patients[54-58]. Additionally, HBeAg that is negatively converted by NUC administration frequently re-appears when NUC administration is stopped (reverse seroconversion)[59,60] after a flare of severe hepatitis[61]. There have been several reports that NUC contributes to HBsAg-negative conversion[62-65]. To improve the long-term prognosis of patients, continuous administration of NUCs for long-term HBV suppression is necessary. Here we focus especially on entecavir and tenofovir disoproxil fumarate (tenofovir), which are now available as first line drugs for chronic hepatitis B in many countries.

Entecavir

Previous reports about entecavir treatment are summarized in Table 4[66-76]. The serum HBV DNA-negative conversion and serum ALT normalization rates of entecavir for 48 to 96 wk were superior to those in response to lamivudine for both HBeAg-positive and HBeAg-negative patients[66,67,70,77]. With entecavir administration for 3 to 5 years, the serum HBV DNA-negative conversion rates were 55% to 88%/1 year, 83% to 93%/2 years, 89% to 95%/3 years, and 91% to 96%/4 years, 94%/5 years; the serum ALT normalization rates were 65% to 84%/1 year, 78% to 88%/2 years, 77% to 90%/3 years, 86%/4 years, and 80%/5 years; and the HBeAg seroconversion rates were 12% to 22%/1 year, 18% to 41%/2 years, 29% to 44%/3 years and 38%/4 years[69,74-76,78]. The resistance mutant emergence rates were reported to be 0.2%/1 year, 0.5%/2 years, and 1.2%/3 to 5 years for the NUCs-treatment-naive patients[69,78].

Today, entecavir is one of the first line NUCs for NUC-treatment-naive patients as well as tenofovir in many countries. However, the serum HBsAg-negative

Table 5 Treatment efficacy of tenofovir in chronic hepatitis B

Ref.	No. of patients	HBeAg	Therapy regimen	HBeAg loss (%)/seroconversion from HBeAg to anti-HBe (%)	Undetectable of HBV DNA (%)	Normalization of ALT (%)	HBsAg loss (*n*)
[1]Marcellin *et al*[83]	176 NUCs - treatment-naive	Positive	TDF 300 mg daily (> 48 wk)	NA/21	76	68	5
	90	Positive	ADF 10 mg daily (> 48 wk)	NA/18	13	54	0
	90	Positive	ADF (48 wk)	NA/18	13	54	0
	125	Negative	ADF (48 wk)	NA/NA	63	77	0
[2]Heathcote *et al*[88]	266	Positive	TDF (> 144 wk)	34/26	71	74	20
	365	Negative	TDF (> 144 wk)	NA/NA	87	81	0
[2]Marcellin *et al*[91]	266	Positive	TDF (> 240 wk)	49/40	65	73	10
	375	Negative	TDF (> 240 wk)	NA/NA	83	85	1

The treatment efficacies were assessed at 48 wk[1], or 144 wk[2]. TDF: Tenofovir; ADF: Adefovir; NA: Not available; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBsAg: Hepatitis B s antigen; Anti-HBe: Antibody to HBe antigen.

conversion is very rare compared with peginterferon and was reported to be 0 to 5.1%/3 to 5 years and 10%/10 years[69,78].

In general, the patients treated with lamivudine and sustained negative HBV DNA are recommended to switch to entecavir or tenofovir. It has been reported that, if the serum HBV DNA level stays negative and there are no resistance mutants during lamivudine administration, entecavir-resistance mutants rarely emerge even when patients are switched to entecavir[79]. Entecavir resistance easily occurs in lamivudine-resistance mutants (rtL180M plus rtM204V) by adding only one more mutation (rt184G, rtS202I or rtM250V) and these states are thought as a low genetic barrier[80]. On the other hand, tenofovir and adefovir lack cross-resistance to entecavir-resistance (rt184G/S, rtS202G/I, M250V)[81]. Therefore, patients who have viral breakthrough under lamivudine administration could easily have entecavir-resistance mutants if they are switched to entecavir, and adding adefovir to lamivudine is generally recommended. Like lamivudine, entecavir was also reported to have a suppressive effect to HCC development compared with the control, and the reported HCC development rates for entecavir *vs* control were 3.7%/5 years *vs* 13.7%/5 years, respectively[10]. The United States Food and Drug Administration (FDA) assigned entecavir to pregnancy category C. Entecavir should not be used for the patients who are co-infected with human immunodeficiency virus (HIV) because of the risk of resistance mutant emergence in HIV.

Tenofovir disoproxil fumarate (tenofovir)

Similar to adefovir, tenofovir is categorized as an acyclic nucleoside phosphonate diester derivative from adenosine monophosphate. Tenofovir has also an antiviral effect against HIV. The usual dosage of tenofovir is 300 mg once a day and higher than that of adefovir 10 mg once a day, owing to the lower nephron-toxicity of tenofovir. The results of tenofovir treatment are shown in Table 5[82-93]. Several reports comparing adefovir 10 mg/d *vs* tenofovir 300 mg/d for NUC-naïve patients reported that the serum HBV DNA suppression rates (< 400 copies/mL) 48 wk after start of administration were 76% *vs* 13% for HBeAg-positive patients and 93% *vs* 63% for HBeAg-negative patients, respectively; also, in general, tenofovir has been superior to adefovir[83]. A study with 144 wk of follow up showed that the serum HBV DNA suppression rates (< 400 copies/mL) were 87% for HBeAg-positive patients and 72% in HBeAg-negative patients at week 144[88]. A 5-year study showed that tenofovir achieved a higher HBsAg-negative conversion rate compared to other NUCs[91].

A recent report with 288 wk of follow up suggested that no apparent resistance mutations were observed[94]. For decompensated cirrhosis, the combination of tenofovir with emtricitabine was reported to achieve positive results[89]. With tenofovir treatment, the serum HBV DNA-negative conversion rate for patients without adefovir-resistance was 100%, but the rate was down to 52% for patients with adefovir-resistance[89]. An important feature of tenofovir is that tenofovir alone or with emtricitabine exerts an anti-viral effect to lamivudine, adefovir or entecavir resistance mutants[85,86,93,95,96]. For example, an article reported that for patients who achieved an insufficient effect by lamivudine, adefovir or the combination of these two drugs, tenofovir resulted in a serum HBV DNA-negative conversion rate of 79%, HBeAg-negative conversion rate of 24% and HBsAg-negative conversion rate of 3% of all patients (the median time from administration to HBsAg-negative conversion was 23 mo)[86]. For patients who achieved insufficient effect by lamivudine and adefovir, tenofovir alone or with lamivudine achieved a 64% serum HBV DNA-negative conversion rate 96 wk after changing therapy[93]. They also reported that they did not observe an obvious resistance mutant[93].

It has been reported that the long-term administration of NUCs improves liver fibrosis. Tenofovir treatment resulted in improvement of the histological findings in 87% of all patients and improvement of liver fibrosis in 51% of all patients[91]. They also reported that 10% of HBeAg-positive patients achieved HBsAg-negative conversion, and most of them were genotype A or D[91]. Tenofovir is only classified as pregnancy category B by the United States FDA.

Table 6 Summary of current and future treatments for hepatitis B virus infection

Current treatments for HBV
Peginterferon therapy
NUCs
Entecavir
Tenofovir disoproxil fumarate (tenofovir)
Future treatments for HBV
NUCs
Tenofovir alafenamide
Treatments for HBV cccDNA
Entry inhibitors targeting of sodium taurocholate cotransporting polypeptide

NUCs: Nucleos(t)ide analogues; HBV: Hepatitis B virus; cccDNA: Covalently closed circular DNA.

Stoppage of entecavir or tenofovir

Apart from interferon, with the use of NUCs such as entecavir and tenofovir, it is always possible for resistant mutants to emerge[97-101]. Suzuki *et al*[101] reported that 51-year-old Japanese women with chronic hepatitis B and cirrhosis have virological breakthrough during combination therapy with tenofovir and entecavir against entecavir-resistant virus. Even long-term therapy with tenofovir against the entecavir-resistant virus has the potential to induce virological breakthrough and resistance. We also reported that virological breakthrough during NUC therapies is also dependent on the adherence to medication[99,100]. In treatment with stronger NUCs, such as entecavir, viral breakthrough associated with poor adherence could be a more important issue[102]. Although we do not know whether durable control of HBV is observed after NUCs are discontinued, NUCs could possibly be stopped in selected patients without causing advanced liver fibrosis.

Adefovir or tenofovir-related Fanconi syndrome is a severe adverse event that results from proximal renal tubular toxicity, which leads to impaired re-absorption of amino acids, uric acid, bicarbonate, glucose and phosphate associated with the increased urinary excretion of these solutes[103-106]. Some cases associated with Fanconi syndrome induced by NUCs-treatment were fully recovered following tenofovir withdrawal[106]. Mitochondrial DNA depletion results in mitochondrial dysfunction in the lamivudine/telbivudine-associated neuromyopathy[107]. During treatment with NUCs, attention should be paid to these adverse events.

FUTURE TREATMENT FOR HBV

Tenofovir alafenamide

Compared with tenofovir, tenofovir alafenamide (GS-7340) is a new tenofovir prodrug, which has demonstrated more potent antiviral activity and lower tenofovir exposures. These might lead to lower nephrotoxicity. Further clinical study will be needed[108-110] (Table 6).

Treatment for HBV cccDNA

In the HBV-infected liver, free HBV DNA and its products are causally related to the activity of liver disease, but the persistence of HBV infection is maintained by the nuclear cccDNA, which serves as a transcription template for HBV mRNA[111,112]. Although there are several opposing views[113], it was reported that HBV cccDNA is noncytolytically degraded by agents that up-regulate apolipoprotein B mRNA editing enzyme and catalytic polypeptide-like (APOBEC) 3A and 3B[23]. In the near future, new therapeutic options to control HBV cccDNA are needed[114-117].

Sodium taurocholate cotransporting polypeptide

Sodium taurocholate cotransporting polypeptide (NTCP) membrane transporter was reported as an HBV entry receptor[118,119]. Iwamoto *et al*[120], Watashi *et al*[121] and Tsukuda *et al*[122] reported that cyclosporine A and its analogs blocked HBV entry through inhibiting the interaction between NTCP and the HBV large surface protein. HBV entry inhibitors might also be useful for controlling HBV infection in the near future.

CONCLUSION

The development of therapies aimed at HBsAg loss, which is the final goal of hepatitis B, is a goal for future research. Further improvements in the therapeutic options for HBV cccDNA are needed.

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REVIEW

Effective treatment strategies other than sorafenib for the patients with advanced hepatocellular carcinoma invading portal vein

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Abstract

Patients with hepatocellular carcinoma (HCC) accompanying portal vein tumor thrombosis (PVTT) have relatively few therapeutic options and an extremely poor prognosis. These patients are classified into barcelona clinic liver cancer stage C and sorafenib is suggested as the standard therapy of care. However, overall survival (OS) gain from sorafenib is unsatisfactory and better treatment modalities are urgently required. Therefore, we critically appraised recent data for the various treatment strategies for patients with HCC accompanying PVTT. In suitable patients, even surgical resection can be considered a potentially curative strategy. Transarterial chemoembolization (TACE) can be performed effectively and safely in a carefully chosen population of patients with reserved liver function and sufficient collateral blood flow nearby the blocked portal vein. A recent meta-analysis demonstrated that TACE achieved a substantial improvement of OS in HCC patients accompanying PVTT compared with best supportive care. In addition, transarterial radioembolization (TARE) using yttrium-90 microspheres achieves quality-of-life advantages and is as effective as TACE. A large proportion of HCC patients accompanying PVTT are considered to be proper for TARE. Moreover, TACE or TARE achieved comparable outcomes to sorafenib in recent studies and it was also reported that the combination of radiotherapy with TACE achieved a survival gain compared to sorafenib in HCC patients accompanying PVTT. Surgical resection-based multimodal treatments, transarterial approaches including TACE and TARE, and TACE-based appropriate combination strategies may improve OS of HCC patients accompanying PVTT.

Key words: Sorafenib; Hepatocellular carcinoma; Portal vein; Thrombosis

Core tip: Given the modest survival gain and the limitation of sorafenib, such as resistance and tolerability, there are still clinical unmet needs in the management of patients with hepatocellular carcinoma (HCC) accompanying portal vein tumor thrombosis (PVTT). Surgical

resection-based multimodal treatments including liver transplantation and transarterial chemoembolization-based appropriate combination strategies for resectable HCC accompanying PVTT may improve overall survival in these patients.

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INTRODUCTION

Globally, hepatocellular carcinoma (HCC) is one of the main reasons of malignancy related death[1,2]. Most HCCs are detected in an advanced stage in spite of surveillance programs for high risk populations, and the prognosis for these patients is poor. Consequently, a minority of patients is eligible for liver resection.

Portal vein tumor thrombosis (PVTT) arises in about 10%-40% of patients at diagnosis[3-5]; lower rates are reported when HCC is diagnosed early usually as a consequence of screening[3] and is apparent in up to 44% of patients with HCC at the end of life[6]. PVTT has a profound adverse effect on prognosis, with the median survival time of patients with unresectable HCC accompanying PVTT being significantly reduced (2-4 mo) compared to those not accompanying PVTT (10-24 mo)[4,5,7]. The range and position of PVTT further affect the prognosis. PVTT is related with poor prognosis probably because of the intensified risk of tumor spread, raised portal pressure inducing variceal bleeding and reduced portal flow causing jaundice, ascites, hepatic encephalopathy and hepatic failure[4,8].

The Liver Cancer Study Group of Japan suggested a macroscopic classification for PVTT: categorized into five grades, Vp0-Vp4 (Figure 1). Each one is defined as follows: no PVTT, Vp0; existence of PVTT not in, but distal to, the 2nd-order branches of the portal vein, Vp1; existence of PVTT in the 2nd-order branches of the portal vein, Vp2; existence of PVTT in the 1st-order branches of the portal vein, Vp3; and existence of PVTT in the main trunk of the portal vein or a portal vein branch contralateral to the mainly involved lobe (or both), Vp4[9]. This classification is helpful, because it is established by surgical outcomes and by the clinical, imaging, and pathological findings.

The presence of PVTT also limits the treatment options, with HCC treatment guidelines often considering PVTT a contraindication for transplantation, curative resection and transarterial chemoembolization (TACE)[10-12]. Current guidelines recommend sorafenib for the patients with HCC with PVTT. Sorafenib is an oral multiple tyrosine kinases inhibitor that suppresses angiogenesis and tumor-cell proliferation and augments the rate of apoptosis[13]. In the Sorafenib HCC Assessment Randomized Protocol (SHARP) study[14] and multicenter study in Asian-Pacific region[15], sorafenib was proved to be efficacious and safe to patients with advanced HCC. Nevertheless, subgroup analyses for macroscopic vascular invasion (MVI) in these two pivotal studies showed only a marginal survival benefit for sorafenib over placebo[16,17]. Therefore, there are still clinical unmet needs in the treatment of patients with HCC accompanying PVTT.

This article review recent data for the various treatment strategies for the patients with HCC accompanying PVTT.

SYSTEMIC THERAPY

HCC is relatively resistant to traditional chemotherapy and liver dysfunction complicates the use of chemotherapeutic agents that undergo hepatic metabolism[8,11]. Sorafenib, a multiple tyrosine kinases inhibitor that blocks tumor angiogenesis and tumor cell proliferation, was the 1st systemic agent proven to significantly increase survival in advanced-stage HCC in randomized controlled trials[14,15]. Sub-analyses of SHARP trial[17] identified 231 patients staged barcelona clinic liver cancer (BCLC) C due to MVI and demonstrated that the sorafenib group (n = 108) achieved a longer median overall survival (OS) (8.1 mo *vs* 4.9 mo) and time to progression (TTP) (4.1 mo *vs* 2.7 mo) than the control group (n = 123) received placebo. In the sub-group analyses of the Asia-Pacific trial[16], patients with MVI and/or extrahepatic spread who received sorafenib (n = 118) showed a better clinical outcome than in placebo group (n = 61): median OS (5.6 mo *vs* 4.1 mo), TTP (2.7 mo *vs* 1.3 mo) and disease control rate (30.5% *vs* 11.5%), respectively. Although the authors argued that the survival benefit with sorafenib was evident regardless of the presence of PVTT in those two pivotal studies, subgroup analyses for MVI showed only a marginal survival benefit of sorafenib over placebo.

LOCO-REGIONAL THERAPIES

TACE

Two key trials and a meta-analysis indicated that TACE can improve survival (median 19-20 mo compared to 16 mo for untreated patients in clinical trials) in intermediate-stage HCC[18-20]. However, PVTT is generally considered a contraindication for TACE because of concerns that interruption to hepatic arterial blood supply could result in an enormous segment of hepatic necrosis in patients whose blood supply is already compromised[8,12]. Nevertheless, there is evidence that selected patients with PVTT can tolerate a modified delivery of TACE provided they have good liver function and collateral blood flow around the obstructed portal vein[4,21]. Recent two studies reported improvements in survival compared to conservative care in HCC patients accompanying PVTT[22,23]. Luo *et al*[22] a performed a prospective nonrandomized study and reported significantly better survival with TACE (n = 84) compared

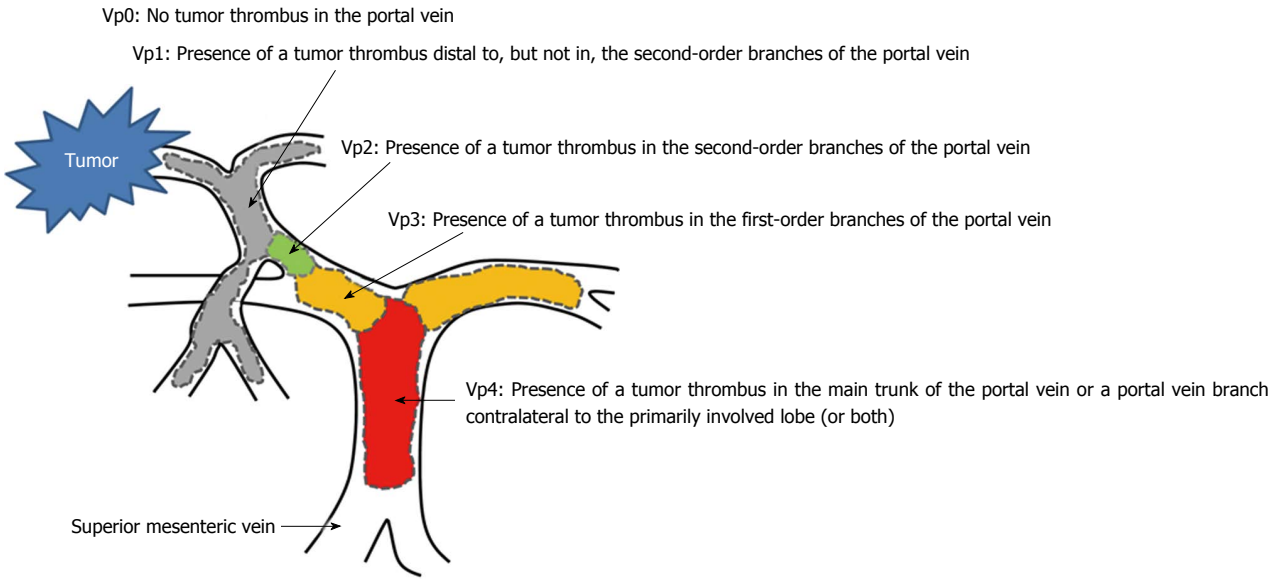

Figure 1 Classification for hepatocellular carcinoma with portal vein tumor thrombosis.

to conservative treatment (n = 80) either in non-cirrhotic or Child A cirrhotic HCC patients accompanying PVTT. The median OS, the 1-, and 2-year survival rates were 7.1 mo, 30.9%, and 9.2% for the TACE arm and 4.1 mo, 3.8%, and 0% for the conservative arm, respectively (P < 0.001)[22]. In the TACE group, the 40 patients with Vp1 or Vp2 survived longer than the 44 patients with Vp3 or Vp4 (median OS 10.2 mo *vs* 5.3 mo)[22]. In the second study, Chung *et al*[23] reported that TACE (n = 83) significantly improved survival compared to supportive care (n = 42; median OS 5.6 mo *vs* 2.2 mo, respectively; P < 0.001) in HCC patients with Vp4. Regardless of treatment (TACE or supportive care), patients with Child class B had worse outcomes (median OS 2.8 mo *vs* 1.9 mo) than those with Child class A (median OS 7.4 mo *vs* 2.6 mo)[23]. In addition, a recent meta-analysis evaluating 8 controlled trials (total 1601 HCC patients) demonstrated that TACE significantly improved the 6-mo (HR = 0.41; 95%CI: 0.32-0.53; P = 0.000) and 1-year (HR = 0.44; 95%CI: 0.34-0.57; P = 0.000) OS of HCC patients accompanying PVTT compared with best supportive treatment[24]. Moreover, another recent study comparing TACE and sorafenib in BCLC stage C HCC patients showed that TACE attained a comparable clinical outcome to sorafenib: the median OS was 9.2 mo (95%CI: 6.1-12.3 mo) for TACE group and 7.4 mo (95%CI: 5.6-9.2 mo) for sorafenib group (P = 0.377)[25]. The proportion of patients who had high-grade adverse events (grade $\geqslant$ 3) was significantly lower in the sorafenib arm (17%) than in the TACE arm (38%) (P = 0.024).

Drug-eluting bead TACE

TACE using DC Bead, drug-eluting microsphere (Biocompatibles UK Ltd, Farnham, United Kingdom), is a relatively novel modality related with favorable systemic doxorubicin exposure/toxicity and liver-specific toxicity compared to conventional TACE[26]. A recent study involving BCLC B HCC patients showed that DC Bead TACE resulted in a significantly better clinical outcome compared to conventional TACE[27]. However, Sellers *et al*[26] reported poor OS in HCC patients accompanying PVTT underwent DC Bead TACE. Further studies are warranted to evaluate the efficacy of DC Bead TACE and sorafenib in HCC patients accompanying PVTT.

Transarterial radioembolization

Transarterial radioembolization (TARE) is a form of catheter-directed, selective internal radiation therapy which delivers 25-32.5 μm sized microspheres loaded with high-energy radioisotope of yttrium-90 (^{90}Y), pure β-ray, into tumor tissue[28]. Tumoricidal radiation doses are delivered with minimal toxicity to functional liver parenchyma and minimal alteration in vascularity with TARE[29,30]. However, there is only microembolization (minimal to moderate embolization)[8,31]. Studies report improved median OS (7-41.6 mo) in BCLC B to C HCC patients following TARE and objective response rates (20%-77%)[32]. Although previous studies reported comparable efficacy for TARE and TACE in terms of tumor response and OS, patients receiving TARE tended to experience fewer complications and fewer days in hospital (typically 0-1.7 d with TARE compared to 1.8-6 d with TACE)[33-36], which are important quality-of-life considerations in patients with unresectable HCC.

Moreover, there is increasing evidence that TARE can be delivered safely and effectively in suitable HCC patients with PVTT, with several studies reporting median OS rates of approximately 10 mo following the procedure in these patients[34,37-42]. Again the extent of PVTT affected survival outcome. Salem *et al*[36] reported that the median OS for patients with Child class A (without extrahepatic spread) ranged from a median 16.6 mo for patients with branch involvement to 7.4 mo for those with Vp4. Median OS in patients accompanying PVTT and Child class B was only 5.6 mo. The risk of

Table 1 Clinical outcomes for hepatocellular carcinoma patients accompanying portal vein tumor thrombosis following surgical resection

Ref.	PVTT status[1]	No. of patients	Survival[2]		
			Median (mo)	1-yr (%)	3-yr (%)
Shi *et al*[49]	Vp2	139	NR	52.1	25.1
	Vp3	169		38.2	17.78
	Vp4	78		24.7	3.6
	Beyond Vp4	20		18.3	0
Lin *et al*[50]	Vp2				
	Vp3	63	NR	52.1	16
	Vp4	5		33.1	0
Chen *et al*[78]	Vp2-4	88	9	31.1	15.2
Matono *et al*[79]	Vp3-4	29	16.9	62.1	24.1

[1]Beyond Vp4 = extending to superior mesenteric vein; [2]Intrahepatic recurred lesions were treated by percutaneous ethanol injection therapy, radiofrequency ablation, transarterial chemoembolization, or systemic chemotherapy based on their hepatic functional reserve and the pattern of intrahepatic recurrence. NR: Not reported; PVTT: Portal vein tumor thrombosis.

death due to underlying liver disease rather than tumor progression becomes a factor in Child class B patients as evidenced by a median OS of only 7.7 mo in the total Child class B cohort despite a TTP of 8.4 mo[43]. Overall, the tolerability of TARE in patients with PVTT appeared to be comparable to that in those without PVTT[37,38,41,42]. When safety issues were specifically investigated, liver decompensation was not observed in the 2-mo period following TARE among HCC patients with PVTT[39], and clinical and laboratory adverse events in the 90-d period after TARE were not more frequent in BCLC C HCC patients than in BCLC A to B HCC patients[38]. Recently, Gramenzi *et al*[44] performed a cohort study directly comparing TARE and sorafenib in patients with intermediate-locally advanced HCC. Median OS of the two groups were comparable even after matching for independent prognostic factors including PVTT: sorafenib group (median OS: 13.1 mo; 95%CI: 1.2-25.9) and TARE group (median OS: 11.2 mo; 95%CI: 6.7-15.7).

Hepatic arterial infusion chemotherapy

The most studies regarding hepatic arterial infusion (HAI) used a combined regimen of cisplatin and 5-fluorouracil. The best results were reported by Ando *et al*[45]. The 5-year OS rate was 11.0% and the median OS was 10.2 mo in that study involving 48 patients treated with Vp2 to Vp4 by HAI with cisplatin plus 5-fluorouracil.

Radiofrequency ablation

In a small sample sized retrospective study (n = 13), radiofrequency ablation could ablaze both single intrahepatic medium-sized (3.7-5 cm) HCCs and the accompanying Vp4 with high efficacy and safety. The 3-year cumulative survival rate was 77%. There were no major adverse events. Mild ascites and elevated transaminase levels were observed in only three patients[46].

Percutaneous laser ablation

In a retrospective study, Lu *et al*[47] evaluated the application of percutaneous laser ablation as a treatment for PVTT in 108 patients and demonstrated that 3 years survival rate was 22.38%.

SURGICAL TREATMENTS

Most patients with HCC with Vp4 are considered technically unsuitable for curative resection, and the presence of PVTT is usually considered a contraindication for liver transplantation due to higher tumor recurrence rates[8]. Surgical resection in HCC patients accompanying PVTT is rare in Occidental area where the BCLC staging system which regards PVTT as a contraindication for surgery is endorsed[8]. However, throughout Oriental area, operation is considered a potentially curative treatment in suitable patients with PVTT as reflected in the consensus recommendations of Asia-Pacific Association for the Study of the Liver[11], although only about 10% of patients undergoing surgery have PVTT[48,49]. Surgical resection in these patients may improve portal venous pressure, liver function, quality of life and survival[8]. The range and position of PVTT significantly affect the potential clinical results following resection[8]. Previous studies have shown that HCC patients accompanying Vp2-Vp3 have better clinical outcomes after resection compared to those with Vp4 or beyond (Table 1)[48-50]. Surgical resection provided survival gains for patients with resectable HCC accompanying PVTT compared with TACE: the 1-, 3-, and 5-year OS rates were 42.0%, 14.1%, and 11.1% for the surgical group and 37.8%, 7.3%, and 0.5% for the TACE group, respectively (P < 0.001)[51]. A sub-group analysis by the PVTT type identified increased survival in the surgical group compared with the TACE group in patients accompanying type Ⅰ PVTT (Vp1-Vp2) or type Ⅱ PVTT (Vp3) (P < 0.001, P = 0.002, respectively)[51]. However, there were no significant differences in OS between the resection group and the TACE group for patients accompanying type Ⅲ PVTT (Vp4) and type Ⅳ PVTT (tumor thrombi involving the superior mesenteric vein) (P = 0.541, P = 0.371, respectively)[51]. In this study, after resection, there was only one postoperative in-hospital mortality caused by postoperative hepatic failure (0.5%), and the

major complication rate was 4.0% (8 of 201). If PVTT is not stick to the portal vein wall, total thrombectomy is possible. However, when the PVTT is adhered to the wall of the portal vein, there is a high chance of intramural invasion of HCC cells into the vessel wall on pathological examination after resection[52]. Therefore, in case of Vp4, the prognosis is extremely poor if the involved wall of portal vein is not resected. Although PVTT is generally considered a contraindication to liver transplantation, some centers have reported their positive results for transplant in the setting of gross vascular invasion. Xu *et al*[53] performed a study involving 24 patients undergoing liver transplantation for HCC accompanying PVTT (10 at main trunk, 10 at right branch, and 4 at left branch) and demonstrated a 6-mo, 1-year, and 2-year OS of 66.7%, 29.5%, and 23.6%, respectively.

EXTERNAL BEAM RADIOTHERAPY

Advances in technology, including three-dimensional conformal radiotherapy, proton beam radiotherapy and stereotactic body radiosurgery, have allowed selective delivery of increased radiation doses to tumors with minimal doses to normal tissue[54]. A number of mostly retrospective studies have examined the use of these new technologies in selected patients accompanying PVTT: median OS (6.7-11 mo), and 1-, 2-, and 5-year survival rates (30%-40%, 20%-30%, and 5.1%-24%, respectively)[55-61]. In a recent retrospective study assessing radiotherapy and surgical resection in 371 resectable HCC patients accompanying PVTT enrolled from two tertiary referral centers, the median OS was 12.3 mo for radiotherapy (n = 185) and 10.0 mo for resection (n = 186). The 1-, 2-, and 3-year OS were 51.6%, 28.4%, and 19.9% for radiotherapy group and 40.1%, 17.0%, and 13.6% for surgical group, respectively (P = 0.029)[62]. More recently, Nakazawa *et al*[63] did a retrospective study comparing the survival benefits of sorafenib *vs* radiotherapy in unresectable HCC patients accompanying PVTT (Vp3 or Vp4). Median OS did not differ significantly between the sorafenib and the radiotherapy group (4.3 mo *vs* 5.9 mo, respectively; P = 0.115)[63]. However, after propensity score matching (n = 28 per group), better median OS was noted in the radiotherapy than in the sorafenib group (10.9 mo *vs* 4.8 mo, respectively; P = 0.025)[63]. In the sorafenib group, 90% (25 of 28) patients permanently discontinued sorafenib owing to disease progression (n = 10) or adverse events (n = 15). However, there was no high-grade (grade $\geqslant$ 3) gastrointestinal or hepatic toxicity in the radiotherapy group. Future large scale prospective studies are warranted to approve the results of these retrospective studies.

COMBINATION STRATEGIES

TACE combined with sorafenib

Zhu *et al*[64] conducted a retrospective study comparing the efficacy and safety of TACE plus sorafenib in 91 HCC patients accompanying PVTT (46 TACE-sorafenib *vs* 45 TACE alone). TACE plus sorafenib showed significant survival benefits over TACE alone in patients with Vp3 (median OS, 13 mo *vs* 6 mo; P = 0.002) or Vp1-2 (median OS, 15 mo *vs* 10 mo; P = 0.003). However, the control arm of this study was TACE alone instead of sorafenib alone. A randomized, controlled phase Ⅲ trial of sorafenib with or without conventional TACE in patients with advanced HCC is recruiting participants (NCT01829035). The result of this study is awaited to answer whether TACE, as a powerful complimentary armament for sorafenib, could be allowed for HCC patients accompanying PVTT.

TACE combined with radiotherapy

The recent advances with a co-treatment modality of TACE combined with radiotherapy have demonstrated superior results over TACE alone[65]. In addition, the survival benefit has been reported in patients accompanying PVTT who have been treated with TACE plus radiotherapy[66-68]. Recently, Cho *et al*[69] conducted a retrospective study comparing TACE combined with radiotherapy (n = 67) with sorafenib (n = 49) in 116 patients accompanying PVTT and demonstrated that OS in the TACE plus radiotherapy group was significantly prolonged over the sorafenib group (14.1 mo *vs* 3.3 mo, P < 0.001). Even in the matched cohort by propensity score, the TACE combined with radiotherapy group demonstrated extended OS over the sorafenib group (6.7 mo *vs* 3.1 mo, P < 0.001)[69].

Surgical resection combined with multimodal treatments

There have been several studies of surgical resection-based multimodality treatment including surgical resection after TACE; surgical resection followed by TACE, HAI, and portal vein infusion chemotherapy; ^{90}Y plus doxorubicin or preoperative intravenous chemotherapy with doxorubicin, cisplatin and 5-fluorouracil plus subcutaneous interferon-α (PIAF); postoperative percutaneous isolated hepatic perfusion; surgical resection followed by interferon with 5-fluorouracil; and surgical resection after radiotherapy. The median OS after surgical resection-based multidisciplinary treatments ranged from 13.0 to 22.1 mo, implying that multimodality therapy contributed to prolonged long-term survival[70-77]. In a controlled trial by Peng *et al*[77], 126 HCC patients accompanying PVTT (Vp3-4) were randomized into TACE after surgical resection (TACE group) or surgical resection alone (control group). The median OS was better in the TACE group (13 mo) than in the control group (9 mo). The estimated survival rates for 1-, 3-, and 5 years were significantly improved in the TACE group (50.9%, 33.8%, and 21.5%; respectively) than in the control group (33.3%, 17.0%, and 8.5%, respectively; P = 0.0094). The available evidence shows that surgical resection-based multimodality treatments are effective and should be estimated in further trials.

Table 2 Comparing various treatment strategies for hepatocellular carcinoma patients accompanying portal vein tumor thrombosis

	Indication	Advantages	Disadvantages
Sorafenib	BCLC stage C	Showing survival benefit in infiltrative type HCC	Modest efficacy compared to placebo control Hand-foot skin reaction
TACE	Nodular type HCC up to Vp4 Child A liver function	Wide indication	Post TACE syndrome Potential risk of liver failure
TARE	Tumor extension ≤ 50% of liver volume Unilobar Nodular type Up to Vp4	Down-staging allowing liver transplantation	Requiring additional lung shunt study due to the risk of lung injury
RFA	Single medium-sized HCCs (3-5 cm)	Less invasive	If the intraparenchymal tumor was not completely ablated by RFA, complete effects on the thrombus probably would not be produced
Surgery	Up to Vp4 Single medium-sized HCCs (≤ 7 cm) Up to Vp4 No HV/IVC invasion	Less expensive technic Better outcomes than other patients with HCC who are BCLC stage C with Child A liver function	Invasive and expensive technic Potential risk of liver failure
External beam radiotherapy	AFP ≤ 30 ng/mL Tumor extension ≤ 60% of liver volume	Combined to multimodal strategies	Potential risk of radiation induced liver disease Potential risk of GI tract toxicities

BCLC: Barcelona clinic liver cancer; HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization; RFA: Radiofrequency ablation; HV: Hepatic vein; IVC: Inferior vena cava; AFP: Alpha-fetoprotein; GI: Gastrointestinal.

INDIVIDUALIZED TREATMENT PLANS FOR DIFFERENT PATIENTS

For HCC patients accompanying PVTT with Child class B, portal hypertension, or Eastern Cooperative Oncology Group (ECOG) 2, sorafenib would be best option as recommended in BCLC guideline. For HCC patients accompanying PVTT with Child class C, portal hypertension, or ECOG > 2, we have to treat these patients with best supportive care. For HCC patients accompanying PVTT with Child class A, no portal hypertension, and ECOG 0-1, we could treat these patients with individualized treatment plans, as follows: (1) Single HCC (≤ 2cm) with PVTT: In this setting, we could consider surgical resection as best options other than sorafenib. Alternatively, TACE and external beam radiotherapy (EBRT) could be other good options; (2) Single HCC (> 2 cm) with PVTT: For single HCC larger than 2 cm with PVTT, we still consider surgical resection as best option for patients with resectable tumor, reserved hepatic function and sufficient post-operative remnant hepatic volume. If tumor size is 10 cm or less, TACE and EBRT could be alternative options. For single huge HCC larger than 10 cm with PVTT, sorafenib would be 1st line option; (3) Multiple (maximal tumor size ≤ 2 cm) with PVTT: If maximal tumor size is 2 cm or less, we could adopt TACE as best option for multiple HCC. Sorafenib would be another best option for these patients; and (4) Multiple (maximal tumor size > 2 cm) with PVTT: In this setting, sorafenib would be 1st line option. However, we could still consider TACE as alternative option if maximal tumor size is 10 cm or less and tumor extent ≤ 50% of liver volume.

CONCLUSION

Although direct appraisals of the clinical outcomes of treatment are inappropriate by the differences in the patients' baseline characteristics (Table 2), in HCC patients accompanying PVTT, evidence from retrospective and prospective studies suggests that multidisciplinary approaches including TACE and/or radiotherapy, TARE, and surgical resection-based multimodal treatments in selected patients may provide better outcomes than sorafenib. For resectable single nodular HCC patients with PVTT, we could treat these patients with surgical resection as 1st line treatment if they have Child class A, no portal hypertension, and ECOG 0-1. TACE, EBRT, and sorafenib would be alternative treatment options for these patients. For multi-nodular HCC patients accompanying PVTT, we could treat these patients with TACE or sorafenib if they have Child class A, no portal hypertension, and ECOG 0-1. TACE would be 1st line if maximal tumor size is 2 cm or less and sorafenib would be 1st line if maximal tumor size is greater than 2 cm. For HCC patients accompanying PVTT with Child class B, portal hypertension, or ECOG 2, sorafenib would be best option. However, for HCC patients accompanying PVTT with Child class C, portal hypertension, or ECOG > 2, we should treat these patients with best supportive care as recommended in BCLC guideline. Given the modest survival gain of sorafenib, surgical resection-based multimodal treatments for resectable HCC accompanying PVTT and TACE-based appropriate combined therapies for unresectable HCC accompanying PVTT may enhance the clinical outcomes of HCC patients with PVTT.

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REVIEW

Update in management of hepatocellular carcinoma in Eastern population

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Abstract

Hepatocellular carcinoma (HCC) is one of the commonest malignant tumours in the East. Although the management of HCC in the West is mainly based on the Barcelona Clinic for Liver Cancer staging, it is considered too conservative by Asian countries where the number of HCC patients is huge. Scientific and clinical advances were made in aspects of diagnosis, staging, and treatment of HCC. HCC is well known to be associated with cirrhosis and the treatment of HCC must take into account the presence and stage of chronic liver disease. The major treatment modalities of HCC include: (1) surgical resection; (2) liver transplantation; (3) local ablation therapy; (4) transarterial locoregional treatment; and (5) systemic treatment. Among these, resection, liver transplantation and ablation therapy for small HCC are considered as curative treatment. Portal vein embolisation and the associating liver partition with portal vein ligation for staged hepatectomy may reduce dropout in patients with marginally resectable disease but the midterm and long-term results are still to be confirmed. Patient selection for the best treatment modality is the key to success of treatment of HCC. The purpose of current review is to provide a description of the current advances in diagnosis, staging, pre-operative liver function assessment and treatment options for patients with HCC in the east.

Key words: Hepatocellular carcinoma; Liver cirrhosis; Treatment; Management; Evidence; Survival; Update

Core tip: Management of hepatocellular carcinoma (HCC) has changed significantly over the past several decades. However, the management of patients has yet to be standardized. As a result of high prevalence of hepatitis B infection in Asia, the experience of the East helped to develop a more aggressive management algorithm. There has been a lot of advancement in terms of diagnosis, management algorithm, staging and treatment methods. This paper will give an update on the management of HCC in the eastern population.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of cancer-related deaths worldwide[1]. Because of the high prevalence of hepatitis B virus infection[2], countries in Eastern and Southeast Asia have the highest incidence of HCC in the world[3]. However, the management of patients with HCC has yet to be standardized in aspects of diagnosis, staging and treatment. Although the management of HCC in the West is mainly based on the Barcelona Clinic for Liver Cancer (BCLC) staging[4], it is considered too conservative by Asian countries. With advancement in diagnosis, staging and treatment, management algorithm of HCC is further modified. The aim of the present review is to provide a summary and update for clinical practice to determine the most appropriate treatment for HCC patients.

DIAGNOSIS

With clinical suspicion or screening, the diagnosis of HCC is based on laboratory tests, radiological imaging and, where appropriate, liver biopsy. The American Association for the Study of Liver Disease (AASLD)[5] diagnostic algorithm is widely used for surveillance and diagnosis. In short, nodules less than 1 cm which cannot be precisely characterised in ultrasound are subjected to interval scan. Nodules detected at ultrasound with diameter greater than 10 mm are further investigated with contrast computed tomography (CT) or magnetic resonance imaging (MRI). Nodules with typical feature of arterial enhancement and porto-venous washout are treated as HCC[6].

MRI was reported to have a higher sensitivity compared with CT[7]. In cases in which the diagnosis is uncertain, a serum alpha-fetoprotein (AFP) level > 400 ng/mL has a high positive predictive value[8]. The sensitivity of MRI scan with contrast ranged from 33% to 61.7% according to different studies targeted for lesions smaller than 2 cm[9]. In order to provide better results, the use of gadoxetic acid-enhanced MRI as an investigation tool has been widely investigated. The initial result of Primovist base MRI showed an improved sensitivity from 64% to 86%[10].

Dual tracer positron emission tomography (PET)-CT with ^{11}C-acetate and ^{18}F-fludeoxyglucose (FDG) markers was reported to have high sensitivity and specificity for diagnosis of HCC. The liver helps to maintain glucose homeostasis[11] and there are a variety of different levels of glucose-6-phosphatase activity and glucose transporters in HCC[12-14]. It was reported that well-differentiated HCCs preferentially accumulate ^{11}C-acetate[15] and poorly differentiated tumours tend to preferentially accumulate FDG. Ho *et al*[15] found dual-tracer PET-CT to have a sensitivity of 98% and a specificity of 86% which were considered significantly improved to other imaging modality alone[16]. Apart from higher sensitivity, the ^{18}F-FDG tracer played an additional role in providing prognostic indicators to lesion with poorly differentiated HCCs[17].

Tumour biopsy can be done if non-invasive studies failed to characterize the lesion.

Diagnostic strategies vary between guidelines, *e.g.,* European Association for the Study of the Liver[18] and AASLD[19], but the process is generally determined by the size of liver nodules. The recently developed technique of dual-tracer PET-CT may help for atypical lesions.

Knowing that in around 30% patients with HCC, the AFP level remains normal, a high index of suspicion is crucial particularly in area like Southeast Asia where hepatitis B is endemic.

STAGING

Cancer staging is the process of determining the extent to which a cancer has developed and help to select the most appropriate treatment for any particular stage of disease[20]. The prognosis and treatment outcome of HCC is related with tumour staging, liver function and patient's physical status[19]. The BCLC staging system[21] and the newly developed Hong Kong Liver Cancer (HKLC) staging system[22] addresses all these factors. A number of other staging systems for HCC are available, *e.g.,* TNM system[23], The Cancer of Liver Italian Program[24], Okuda staging system[25], *etc*., but they only included some of these related factors.

In Eastern Asia where the highest incidence rates (> 20/100000 per year) of HCC occur, hepatitis B infection accounts for 70% of HCC cases[26]. While in Europe and North America where lower incidence rates (< 5/100000 per year) of HCC, hepatitis C and alcohol are the major etiologies. In view of the difference in epidemiologic and clinical characteristics, different therapeutic approaches were developed in the East and West centres[4,22,27,28]. Indeed, until recently, studies comparing treatment outcomes of eastern and western experiences with HCC are lacking.

The BCLC is widely used and quoted in the literature. However, it was derived from analysis of cohorts involving mainly Caucasian and may work better in Caucasian populations[29-31]. In Asia, more aggressive treatment options were recommended especially for BCLC intermediate- and advanced-stage patients[32,33]. The differences of treatment algorithms of APASL guideline, Japan Society of Hepatology guidelines, HKLC and BCLC staging systems were summarized in Table 1. These guidelines are followed by the corresponding community. However, the comparison between treatment outcomes following different algorithms is subjected to future study.

Recently, a new HCC classification system has been proposed in Hong Kong in order to provide a better guideline in area where the proportion of HCC and cirrhosis is higher. Although BCLC staging had fairly good discriminatory power in the test set, HKLC staging was significantly better than BCLC staging statistically in stratifying HCC patients into different prognostic groups. Overall, our HKLC treatment algorithm yielded

Table 1 Comparison between treatment algorithms of Asian guidelines for hepatocellular carcinoma and Barcelona Clinic for Liver Cancer staging system

	The HKLC staging system[22]	The JSH guidelines[28]	The APASL guidelines[27]	The BCLC staging system[4]
Parameters included	Performance status Liver function Vascular invasion/metastases Tumour staging	Liver function Vascular invasion/metastases Tumour staging	Liver function Vascular invasion/metastases Tumour staging	Performance status Liver function Vascular invasion/metastases Tumour staging
Definition of vascular invasion	Extrahepatic vascular invasion: main portal vein and inferior vena cava invasion	Portal vein invasion categorized into Vp1-4	Invasion to hepatic/portal vein branches	Portal vein invasion considered as advanced stage
Definition of tumour staging	3 categories: early, intermediate, locally advanced	Categories according to number and size	3 categories: resectable, non-resectable within Milan criteria, non-resectable exceeding Milan criteria	5 categories: very early, early, intermediate, advanced and terminal stages
Criteria for resection	Early tumour, Child A/B and intermediate tumour Child A Left or right portal vein invasion can be considered for resection	Any resectable HCC	Resection can be considered for number ⩾ 4 although TACE is the first choice HCC with portal invasion at second or more peripheral portal branch can be considered for resection	Only solitary HCC or 3 nodules < 3 cm are subjected to resection

HKLC: Hong Kong Liver Cancer; JSH: Japan Society of Hepatology; BCLC: Barcelona Clinic for Liver Cancer; HCC: Hepatocellular carcinoma; TACE: Transarterial chemoembolization; APASL: The Asian Pacific Association for the Study of the Liver.

better survival outcomes when compared with the BCLC treatment algorithm, as evidenced by the hypothetical survival curves. The effectiveness of the HKLC treatment guidelines *vs* the BCLC treatment schedule has been clearly observed in the aforementioned patient subsets.The Hong Kong group showed better survival outcomes can be achieved if these patients received a more radical approach of therapies[22].

LIVER RESECTION

Pre-operative patient evaluation and case selection

Liver resection follows the basic principle of surgery. The patients have to be fully assessed for performance status and anaesthesiology fitness together with the tumour status including the tumour locations. Although BCLC criteria do not suggest liver resection for Stage B disease which comprise of tumour larger than 5 cm in size, many centres in Asia does not consider tumour size as an absolute contraindication to surgery. In many of the Asian centres including Hong Kong will consider hepatectomy for HCC as long as there is no extrahepatic disease or multifocal diffuse disease[34].

The other factor that affects liver resection is liver function reserve assessment. More than 80% of patients with HCC are hepatitis B carrier and around half of these patients have different degree of cirrhosis. It is important that patients with poor liver function reserve should not receive excessive removal of liver parenchyma in order to avoid postoperative liver failure. In general only selected patients with Child A cirrhosis should be considered for major hepatectomy[35]. In addition, several adjuvant investigations are crucial as part of preoperative liver function assessment.

Indocyanine green clearance test: Indocyanine green (ICG) is a dye which binds completely to albumin and β-lipoprotein and is exclusively removed by the liver and excreted unchanged in bile without any entero-hepatic circulation[36]. The ICG retention at 15 min (ICGR-15) can be measured with serial blood sampling or pulse spectrophotometry methods[37,38]. ICGR-15 is about 10% in normal person. It was shown to be correlated with hospital mortality so that it was recommended that ICGR-15 for a safe major and minor hepatectomy are 14% and 22%, respectively[35].

Imaging-based volumetry: Liver volumetry is most commonly estimated with 3-D volume CT calculation[39]. The post-operative residual liver volume [future liver remnant (FLR)] can be calculated based on cross-imaging techniques, on each slice FLR are outlined and integrated[40]. The estimated standard liver volume (ESLV) can be calculated with a formula based upon regression analysis of normal population in which body weight and height are included[41,42]. The FLR-to-ESLV ratio was shown to have inverse correlation with increasing risk for post-hepatectomy liver failure and post-operative death.

The critical residual liver volume for patients with normal liver had been reported to be 20%-30% according to different authors[43-47]. In general FLR > 20% is considered safe and with low risk of postoperative hepatic dysfunction[46]. However, most HCCs are associated with cirrhotic liver or diseased liver. The safety of surgical resection is greatly determined by the degree of liver dysfunction due to the underlying liver disease[48,49]. In literature, it is generally accepted that the FLR required is considerably larger than those with a normal liver, given the impaired baseline function of hepatocytes. It was

reported that FLR > 30%-50% are considered safe for patients with diseased liver[50-52].

SURGICAL RESECTION

The aim of hepatectomy is to obtain radical resection with adequate liver reserve. When performed in specialised centres, hepatectomy can achieve 5-year survival above 50%[53]. In the more aggressive APASL guideline, the only contraindication to resection is the presence of distant metastases, main portal vein or inferior vena cava involvement[27]. Even for advanced tumour > 5 cm or multinodular (> 3 nodules), a 5-year survival of 39% was reported[34] comparing with 5-year survival for transarterial chemoembolisation (TACE) was only 6%-19%[54-56]. While following the BCLC protocol, patients with multinodular tumours would be excluded for surgical resection. Vascular invasion was associated with poor prognosis in untreated patients[4]. As liver transplant is contraindicated and TACE or systemic therapy is ineffective, surgical resection remains the only possible curative treatment in patients with good liver reserve. Five years survival of patients with portal vein thrombus underwent liver resection was reported to be 26%-42%[57,58].

Conventionally, liver resection is mainly carried by open approach. In recent years, laparoscopic liver resection for cancer has gaining popularity and many results showed that minimally invasive approach can produce equally good oncological outcome even in patients with liver cirrhosis for minor hepatectomy[59].

Laparoscopic hepatectomy was initially adopted to treat peripheral, benign tumour in a normal liver. Multiple series showed the feasibility of its application for HCC[60]. However, patient selection needs to be careful. Some technical manoeuvres frequently used in open hepatectomy, such as organ mobilization, control of vascular inflow, and hanging manoeuvre are difficult in laparoscopic setting and controlling haemorrhage is also difficult. The Louisville consensus suggested that the laparoscopic approach to left lateral sectionectomy should be considered standard practice[61].

PORTAL VEIN EMBOLISATION AND THE ASSOCIATING LIVER PARTITION WITH PORTAL VEIN LIGATION FOR STAGED HEPATECTOMY

Hepatectomy is the only option for long term survival for many patients with HCC. However, the resectability rate for HCC is approximately 20%-30% with normal liver, and further reduced in patients with cirrhotic liver[62]. Portal vein embolisation (PVE) is one of the methods to stimulate growth of the FLR. Kinoshita *et al*[63] reported the first preoperative PVE in 1986. Various techniques for PVE were reported and percutaneous transhepatic technique has become the standard technique for PVE. The mean period between PVE and hepatectomy was reported to be 37 d (range: 21-84 d) and the mean hypertrophy rate of FLR was reported to be 38%[64]. On the other hand, approximately 10% of patient cannot have the surgery performed because of failure of PVE, inadequate hypertrophy, complication leading to unresectability and local tumour progression[64].

The associating liver partition with portal vein ligation for staged hepatectomy approach associates in-situ splitting to portal vein ligation. It has been shown to be effective for the induction of rapid FLR hypertrophy so as to improve the respectability[65]. It was proposed that the portal flow deprivation in future resected liver and accentuated inflammatory response induces faster regeneration compared to traditional portal vein occlusion methods[66]. Therefore, the approach may reduce dropout in patients with marginally resectable disease but the midterm and long-term results are still to be confirmed.

LOCAL ABLATION THERAPY

Local ablation therapy is used in patients with early-stage HCC who are not suitable for surgical resection[67]. The currently preferred methods included radiofrequency ablation, microwave ablation and High Intensity Focused Ultrasound (HIFU).

Radiofrequency ablation (RFA) utilises alternating electrical current with a frequency 200 k-20 MHz in the range of radio-waves and causes coagulative necrosis and tissue desiccation[68]. RFA has largely replaced percutaneous ethanol injection because it produces better recurrence-free survival and requires fewer treatment sessions[69]. RFA can be performed percutaneously under image guidance or during surgery guided by intraoperative ultrasound. Complete ablation of small lesions is possible in more than 90% of cases[70]. The overall 5-year survival rates between 33% and 55% in selected series[71]. and lower mortality and morbidity rates were reported[72]. Therefore, RFA is considered as an alternative to resection especially for small HCC. Three randomized controlled trials are available comparing hepatic resection and RFA[73-75]. Although they reported conflicting results, it seems reasonable to offer RFA to very small HCC (< 2 cm) with no technical contraindications[76]. Except for the use of RFA on solitary tumours, multifocal tumour prevalence was also high in ablated patients. Rather than competing techniques, RFA can sometimes be combined with hepatectomy tailored to suit the anatomical condition.

Microwave ablation (MWA) utilizes electromagnetic methods for tumour destruction with frequency $\geqslant$ 900 MHz[77]. Microwave ablation can achieve higher temperature[78] shorter ablation time[78-81] and does not require the placement of ground pads. Several studies[82-84] showed that the local tumour control, complications and long-term survival were equivalent for RFA and MWA in the treatment of HCC.

HIFU is a non-invasive modality that uses an

extracorporeal source of focused ultrasound energy[85]. It is able to induce coagulative necrosis in selected tissues. HIFU ablation utilizes a unique frequency of ultrasound wave of 0.8 to 3.5 MHz, which can be focused at a distance from the therapeutic transducer. The accumulated energy at the focused region induces necrosis of the target lesion by elevating the tissue temperature to above 60 ℃. Temperature outside the focus point remains static as particle oscillation remains minimal. As ultrasound energy travels much better in water than in air, the presence of ascites in HCC patients actually facilitates energy propagation to the target HCC. Passage of energy without the puncture of a physical instrument and its superior performance in patients with ascites give HIFU ablation superiority over other treatment modalities for HCC. Initial results of HIFU ablation in the management of HCC were promising with a complete ablation rate of 28.5% to 68% for single treatment[86]. This non-invasive approaches has shown to produce very little collateral damage to the normal liver parenchymal in patients with cirrhosis and is well tolerated even in selected patients with Child Pugh C liver cirrhosis. HIFU was also shown to be safe and effective to reduce the drop-out rate of liver transplant candidate[87].

LIVER TRANSPLANTATION

Liver transplantation treats HCC together with the underlying liver disease. The Milan criteria (single tumour $\leqslant$ 5 cm, up to 3 tumours each $\leqslant$ 3 cm in diameter) is the gold standard for selection of deceased donor liver transplantation (DDLT). The disease-free survival at 4 years for patients within the criteria was reported to be 92%[88]. Many centres extended the criteria as evidence showed that patients outside the Milan criteria also have favourable outcomes after liver transplantation. In the west, the University of California, San Francisco criteria (single tumour $\leqslant$ 6.5 cm, up to 3 tumours at most with the largest $\leqslant$ 4.5 cm, and total diameter $\leqslant$ 8cm) had been shown with comparable outcome with Milan criteria[89]. Asian centres extended the HCC transplant criteria further because the majority of liver grafts were from living donor[90-93]. Living donor organ is considered a "gift" and there is less societal concern of equity[94].

In Asia, the organ donation rates remain the lowest worldwide[90]. As a result, living donor liver transplantation (LDLT) comprises the major workload and becomes an important treatment option for managing HCC in Asia. The overall survival rates were shown to be the same with studies comparing outcomes of DDLT and LDLT[95-99].

In view of the shortage of donated organ in Asia, multiple strategies had been developed, including using marginal livers, domino donors, and split liver transplant. Also bridging therapy with ablative and transarterial interventions aims to prevent tumour progression[100]. However, in survey up to 2005, 96% of liver transplant for HCC in Asian centers were from live donors[90].

TRANSARTERIAL LOCOREGIONAL THERAPY

TACE is the most widely used treatment for HCCs which are unresectable or cannot be effectively treated with percutaneous intervention for over 3 decades[54,101,102]. During the procedure, iodized poppy seed oil (lipiodol) and chemotherapeutic agents are administered through the feeding artery of the tumour, followed by arterial embolization. TACE results in delay tumour progression and vascular invasion and result in a survival benefit compared with conservative management. The most important aspect is the selection of patients, *i.e.*, patients should have preserved liver function, with no portal vein thrombosis and extrahepatic spread. A meta-analysis of six trials found a survival benefit for TACE over conservative management[101]. Two-year survival rates were reported as 31% *vs* 11% for conservative treatment[54]. TACE can be combined with other ablative therapies such as RFA[103,104].

The use of drug eluting bead (DEB) in TACE was reported in 2007[105,106] for its safety and efficacy. Microspheres composed of synthetic polymers or natural materials such as albumin, gelatine, chitosa or aliginate roughly fall into two categories - 15-60 μm and 100-250 μm[107]. Drugs included doxorubicin, mitomycin C, cisplatin, *etc*., can be loaded to the beads[107]. DEBs have potential to simplify and standardize the TACE procedure by preloading the embolic with drug followed by controlled drug elution in target tissue[107]. A randomized controlled trial in 212 patients with HCC demonstrated that DEB-TACE is better tolerated than conventional lipiodol-based TACE, but this trial failed to demonstrate superiority in tumour response[108].

Transarterial radioembolization (TARE) using yttrium-90 microspheres can be used for patients with portal vein thrombosis[109-115]. Tumoricidal radiation doses are delivered with minimal toxicity to functional liver and there is minimal to moderate embolization (micro-embolization) and minimal alteration in vascularity with TARE. Several authors have compared outcomes following TACE with TARE in matched patient cohorts, and reported comparable efficacy for TACE and TARE in terms of tumour response and overall survival[116-119]. However, randomized controlled trials comparing their efficacy with other therapies are lacking[120].

SYSTEMIC THERAPY

Systemic chemotherapy had a disappointing record in management of HCC[121]. With recent knowledge of hepato-carcinogenesis, there has been encouraging development in target therapy of advanced HCC. Sorafenib is an oral multikinase inhibitor that has activity against several serine/threonine kinases and tyrosine kinases. It was the first systemic therapy shown to prolong survival in patients with HCC, and is approved for use in advanced HCC[122]. The Sorafenib HCC Assessment Randomized

Protocol trial[123] is a large, placebo-controlled phase Ⅲ trial in patients with advanced HCC and preserved liver function (Child-Pugh class A). It demonstrated prolonged median survival for approximately 3 mo in sorafenib group. Cheng *et al*[124] in another study involving 271 Asian patients showed a 2 mo prolongation in survival in patients with advanced HCC. Many other novo agents were being investigated at the moment but it was only Sorafenib that has demonstrated the effect of providing significant longer overall survival.

CONCLUSION

Management of HCC has changed significantly over the past several decades. However, the management of patients has yet to be standardized. As a result of high prevalence of hepatitis B infection in Asia, the experience of the East helped to develop a more aggressive management algorithm. To ensure the most effective treatment to be offered for HCC patients, a good patient selection for the right modality need to be practised.

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MINIREVIEWS

Th1/Th2 cytokines and their genotypes as predictors of hepatitis B virus related hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC), the predominant type of primary liver cancer, is one of the most serious life-threatening malignancies, worldwide. In majority of the cases, HCC develops after prolonged and persistent chronic liver disease. hepatitis B virus (HBV) or HCV infection is prominent etiological factors, attributing to this condition. It has been well documented that HBV, being the inducer of chronic inflammation, is the main causative agent in causing HCC, particularly in Asian countries. The HBV infection leads to a wide range of clinical symptoms from carrier state to malignancy. Cytokines being immune-modulatory molecules, are the key mediators in the defense mechanism against viral infection. In this regard, this review will detail the substantial role of key Th1: interleukin 1 (IL-1), IL-2, IL-12, tumor necrosis factor-α, interferon-γ; Th2: IL-4, IL-10 and non Th1/Th2: IL-6, transforming growth factor-β1 cytokines genotypes in analyzing the variability in the clinical manifestations in an HBV-afflicted individual, which might finally, culminates into HCC. Since cytokine production is regulated genetically, the cytokine promoter region single-nucleotide polymorphisms induced changes, greatly affects the cytokine production, thus resulting into differential outcome of immune balance.

Key words: Hepatitis B virus; Hepatocellular carcinoma; Inflammation; Th1/Th1 cytokine; Polymorphism

Core tip: Hepatocellular carcinoma is the prime manifestation of primary liver cancer. Besides, hepatitis B virus (HBV) infection accounts for nearly 50% of hepatocellular carcinoma cases worldwide. The injuries afflicted by HBV infection are predominantly immune-mediated. Th1/Th2 cytokines play a significant role in modulating almost all phases of the host immune response. Moreover, cytokine production and response is genetically controlled. Hence, the population-based variability in patterns of cytokine polymorphisms, might alter the ability of an individual to mount an appropriate immune response, thus causing a differential effect on the progression of the HBV disease pathogenesis.

Saxena R, Kaur J. Th1/Th2 cytokines and their genotypes as predictors of hepatitis B virus related hepatocellular carcinoma.

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INTRODUCTION

Liver cancer includes a wide array of histologically different primary liver cancers comprising hepatocellular carcinoma (HCC), cholangiocarcinoma, hepatoblastoma, bile duct cystadenocarcinoma and haemangiosarcoma. However, out of all these, HCC, a type of hepatocyte epithelial tumor, is the most common, constituting 83% of all the incidences[1,2]. Additionally, HCC is one of the virus-induced human cancers[3].

HCC, poses as a worldwide public health issue, being one of the most widespread and lethal cancers. Accounting for 85%-90% of primary liver cancers[4], HCC is the third most frequent mortality causing malignancy[5]. Roughly 6% of the existing human cancers are HCC induced. The occurrence of over half a million HCC cases, annually worldwide[6], makes it the fifth most widespread cancer (fifth in men and seventh in women), globally[7,8].

The major fraction of the HCC afflicted patients, occur due to infections with hepatitis B virus (HBV) or HCV, constituting the main agents, attributing to this condition. This is primarily due to their role in induction of chronic inflammation. However, out of these two causative agents, HBV is regarded as the predominant causative factor of HCC, worldwide, with the incidence rate of hepatitis B surface antigen (HBsAg) carriers accounting for nearly 2% to 11% of the Indians[9]. The variability in HBV infection induced response, is partly due to different immunological factors like the innate and adaptive immune response against the viral infection. Besides, HBV being a non-cytopathic virus, viral persistence/clearance following HBV infection, occur due to the body's immune response against viral antigens.

HBV: Major causative factor for HCC

HBV infection is considered to be one of the pivotal factor in causation of HCC, with the occurrence of more than 350 million chronic carriers worldwide. HBV has been declared a human cancer causing agent by International Association for Research on Cancer, in 1994. Besides, the recent Asian and Northern-American studies conducted, estimated that the chances of HCC development increases by 25-37 times in HBsAg carriers as compared to control populations[10]. India, one of the most populous developing countries has about 45 million chronic-HBV afflicted people[11]. Numerous reports has suggested that the HBV is not directly cytopathic and hence, any injury to the liver cell is chiefly governed by cytotoxic T cells[12]. A large body of evidence has demonstrated that liver cell injury resulting from chronic immune response triggers the causation of HCC. Moreover, cell-mediated immune responses' induced chronic hepatic inflammation and regeneration, cause the accumulation of genetic alterations in infected liver cells[3]. Thus, these findings strongly reflect the role of immune responses following HBV infection, in causing the chronic disease to carcinoma. Also, all the other procarcinogenic events leading to HCC, most likely occur due to this process[13]. Therefore, the probability and intensity of the hepatocyte injury and its further progression to cirrhosis and consequently to HCC, is an outcome of the interplay between the host immunity and the virus replication ability[14].

CYTOKINES

Cytokines are proteineous moieties, produced chiefly by immune/non-immune cells[15]. They are potent immune-modulatory molecules and major players in protection against viral infection, by either analyzing the host response pattern or by inhibiting viral replication[16].

Since cytokine production is controlled genetically, variations caused due to single-nucleotide polymorphisms (SNPs) in cytokine genes' promoter region, affect the cytokine production to a great extent, thus affecting the immune balance response. This might hold true for cytokine gene polymorphisms and the HBV related HCC, as liver is an lymphocyte enriched organ, involved in numerous cytotoxic activities and having variable cytokine secretion patterns. Besides, HBV is widely believed to be strongest inducer of HCC, primarily by inducing chronic inflammation. Though, some earlier studies have been carried out in this regard, which have reported variable results concerning association of cytokine polymorphism/expression with HBV-HCC risk in different ethnic groups, but till date, no substantial evidence has been yet obtained from the Indian population.

Though, initial classification divided the cytokines into four large groups, on the basis of their biological functions[17]: (1) Natural immunity mediators: like tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), IL-6 (minor role), IL-5, IL-8 and the chemokines; (2) Lymphocyte activation, growth and differentiation regulators: like IL-2, IL-4, transforming growth factor-β (TGF-β); (3) Regulators of Immune-mediated inflammation: IL-4, TGF-β, IL-10, IL-1, interferon-γ (IFN-γ), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage activating factor; and (4) Stimulators of immature leucocyte growth and differentiation: IL-1, IL-3, IL-5, IL-6, granulocyte-CSF, macrophage-CSF, GM-CSF.

However, due to the overlapping and multifunctional nature of many of these cytokines, this classification is considered to be random[15]. So, these are generally catagorized into two groups[18]: (1) Th1 (pro-inflammatory) cytokines: IL-1, IL-2, IL-12 and non-ILs like TNF-α and IFN-γ. These cytokines cause stimulation of virus-specific CD8-positive cytolytic T lymphocytes, leading to viral clearance; and (2) Th2 (anti-inflammatory) cytokines: IL-4, IL-10. They induce Th1 cytokines and stimulate activation/differentiation of B cells. Although

Table 1 Cytokines involved in hepatitis B virus - hepatocellular carcinoma risk

Cytokine genes	Physiological function	Role in viral clearance/persistence	SNP analyzed	Disease association
IL-1	Proinflammatory	Viral clearance	IL-1B (-511 C > T)	NS[28,29,31]; risk[30]
			IL-1RN (VNTR)	Risk[28]; protection[29];
			Intron 2	NS[30,31]
			IL-1 haplotypes	Protection[28]
IL-6	Pro- as well as anti-inflammatory	Both	-572 C > G	Protection[38]; NS[39]
			-597 G > A	Protection[38]
			IL-6 haplotypes	Risk[38]
IFN-γ	Proinflammatory	Viral clearance	+874 T > A	Protection[42]; NS[43]
IL-10	Anti-inflammatory	Viral persistence	-819 C > T/-592 C > A	Risk[49]; NS[50]
			IL-10 haplotypes	NS[49,51]
IL-12B	Proinflammatory	Viral clearance	+1188 A > C 3'UTR	NS[50,54,55]
TNF-α	Proinflammatory	Viral clearance	-308 G > A	NS[42,60]; risk[59]
TGF-β1	Pro- as well as anti-inflammatory	Both	-509 C > T	Risk[54]; protection[54,70,71]
IL-2	Proinflammatory	Viral clearance	-330 T > G	NS[50,54,77]; risk[78]
IL-4	Anti-inflammatory	Viral persistence	-590 C > T	Protection[54]; NS[50]

NS: Non-significant; TNF-α: Tumor necrosis factor-α; IL: Interleukin; TGF-β: Transforming growth factor-β; VNTR: Variable number tandem repeat; IFN-γ: Interferon-γ; 3'UTR: 3' untranslated region.

several of them do not fit specifically into either category like non Th1/Th2 cytokines; IL-6, TGF-β. Although cytokines act at very low concentrations (pg/mL), their effect is closely related to their circulating levels. Besides, an individual's cytokine production capacity is genetically regulated, which accounts for remarkable variation among individuals[19]. Thus, deregulation of the gene expression that alters the cytokine production may alter the homeostasis of the organism, resulting in organ-specific or systemic failures. This is quite relevant for cytokine gene polymorphisms and HCC[20], as cytokines are key determinants in regulating the immune response during HBV infection.

Role in HBV-HCC pathogenesis

Induction of chronic inflammation creates a tumor-favouring microenvironment that eventually participates in the necroplastic process. Moreover, in immune cell enriched liver, immune responses following hepatitis infection, cause cell damage, regeneration, finally leading to liver cancer due to continued cell proliferation and death[18]. Thus the chances for HCC development in HBV-afflicted individuals increases with up-regulated inflammation and fibrosis[21]. T-lymphocyte immuno-regulatory cytokines are crucial players in regulation of the host response to against HBV infection. In fact, it has been shown that the cell-mediated immunity is responsible for viral recovery[22], while Th2 cytokines actively participate in causing persistent infection[23]. In this context, an HBV-infected individual having down-regulated Th1 and up-regulated Th2 cytokine production, might experience an increased likelihood to HCC development. Hence, polymorphisms in cytokine genes can influence body's immune system; inflammation and tissue injury in HBV related malignancy.

Several studies have documented functional cytokine polymorphisms, associated with varying stages of liver disease. The differences in cytokine expression and the functional consequence of these modifications in HCC, are primarily the result of the variability in response of the immune system in the presence of primary lesion. However, the genetic make-up of an individual may also alter the immune system and generate tumorigenic effects. The principal cytokines and their genotypes, found to be involved in HBV-HCC development are listed below.

IL-1

This is a multifunctional proinflammatory cytokine. The *IL-1* gene family comprises IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra/IL-1RN). It is located on long arm of chromosome 2 (2q13.21)[24] and encodes three proteins namely: IL-α, IL-β (agonists) and IL-1Ra (naturally occurring inhibitor)[25]. An 86-bp variable number tandem repeat (VNTR) polymorphism is present in intron 2 of the *IL-1RN* gene[26]. The *IL-1RN* (VNTR) polymerase chain reaction-analysis, depicted five different allelic combinations (allele 1 - allele 5) of the 86-bp sequence to be present in intron 2 of the *IL-1RN* gene. Pociot *et al*[27] have identified an *IL-1B* biallelic (C/T), promoter region polymorphism (-511), affecting its secretion *in vitro*.

The *IL-1B* (-511) genotypes and HBV-HCC association analysis (Tables 1 and 2), revealed that there was no significant association between the *IL-1B* (-511) heterozygous (CT) and variant (TT) genotypes with HBV-HCC risk, in healthy controls and inactive-HBV carriers[28]. Similarly, a study by Zhang *et al*[29] indicated no change in IL-1B allele/genotype frequencies between hepatitis patients and the controls. These findings, however, differed from a study by Tanaka *et al*[30], where *IL-1B*-511(TT) genotype was potentially in positive association with HCC development (Table 1). Further, we observed that the *IL-1RN* (VNTR) genotypes and the HCC risk association analysis (Tables 1 and 2), revealed a significant positive association between 1/2 genotype with HCC development, among healthy controls and inactive carriers[28]. However, a study by Zhang *et*

Table 2 Association of various cytokine genotypes in progression of hepatitis B infection

Cytokine genes	OR (95%CI)					Ref.
	Control	Inactive HBV-carrier	Chronic-active HBV	HBV-cirrhotic	HBV-HCC	
IL-1RN (VNTR) 1/2	1 (REF)	0.45[a] (0.2-1)	2.70[b] (1.3-5.3)	2 (1-4)	1.90 (1-4)	[28]
	-	1 (REF)	5.80[h] (2.5-13.4)	4.20[f] (1.8-10)	4.12[f] (1.7-10)	
IL-6 (-572 G > C) GC	1 (REF)	3.98[b] (1.5-10.2)	2.50[a] (1.1-6)	0.94 (0.4-2)	0.75 (0.3-1.7)	[38]
	-	1 (REF)	0.63 (0.2-2)	0.24[f] (0.1-0.7)	0.20[f] (0.06-0.6)	
CC	1 (REF)	1.8 (0.6-5.6)	2.54[a] (1.05-6.2)	0.40[a] (0.16-1)	1.50 (0.6-3.8)	
	-	1 (REF)	1.30 (0.3-5)	0.20[c] (0.01-0.6)	0.83 (0.2-3.2)	
IL-6 (-597 G > A) GA	1 (REF)	8.65[d] (3-25)	0.52 (0.2-1.2)	0.63 (0.3-1.5)	2.1 (0.7-6.4)	
	-	1 (REF)	0.06[h] (0.02-0.2)	0.07[h] (0.03-0.2)	0.22[c] (0.06-0.8)	
IFN-γ (+874 T > A) TA	1 (REF)	2.20 (0.6-8)	0.34[b] (0.14-0.8)	0.56 (0.24-1.3)	0.39[a] (0.17-0.85)	[42]
AA	1 (REF)	0.65 (0.26-1.7)	0.78 (0.34-1.8)	0.62 (0.26-1.5)	0.31[b] (0.13-0.72)	
IL-10 (-819 C > T/-592 C > A)	1 (REF)	4.34[d] (1.83-10.3)	ND	2.02[a] (1-4.1)	2.20[a] (1.05-4.5)	[49]
IL-12B (+1188 A > C 3'UTR) CC	1 (REF)	1.44 (0.5-4.1)	3.30[b] (1.3-8)	1.3 (0.5-3.8)	1.80 (0.6-5.3)	[54]
TGF-β1 (-509 C > T) CT	1 (REF)	4.70[d] (1.8-12)	2.20[a] (1-4.5)	2.81[b] (1.3-5.8)	2.10[a] (1-4.2)	
TT	1 (REF)	15.42[d] (5-47.6)	5.87[d] (2.2-15.7)	1.50 (0.4-4.8)	3.72[b] (1.4-10)	
	-	1 (REF)	0.38 (0.13-1.1)	0.10[b] (0.03-0.3)	0.24[f] (0.1-0.7)	
IL-4 (-590 C > T)	1 (REF)	2.26[b] (1.2-4.2)	0.40[b] (0.2-0.7)	0.70 (0.38-1.27)	1.65 (0.9-3)	

OR: Odd ratio adjusted with age, sex, bilirubin, total protein, A/G, aspartate transaminase, alanine transaminase, alkaline phosphatase; ND: Not determined due to a single subject having this genotype. [a]$P < 0.05$, [b]$P < 0.01$, [d]$P < 0.001$ with respect to control; [c]$P < 0.05$, [f]$P < 0.01$, [h]$P < 0.001$ with respect to inactive HBV-carrier. HBV: Hepatitis B virus; IFN-γ: Interferon-γ; IL: Interleukin; TGF-β: Transforming growth factor-β; HCC: Hepatocellular carcinoma.

al[29], documented conflicting results, by showing a significant negative association of the carriage of *IL-1RN* (VNTR) allele 2 with HBV infection. On the contrary, a non-significant association was evident between 2/2 genotype and the liver disease progression in a Japanese study[30], while a potential association was found between the same genotype and cirrhosis development, in our case[28]. Moreover, as reported by Chan *et al*[31], no significant association was found between *IL-1B* and *IL-RN* (VNTR) polymorphisms and liver fibrosis, in Chinese hepatitis patients. Besides, we found that the *IL-1* haplotypes 2 and 3 acted as significant protective factors for hepatitis and subsequently for HCC development (Table 1)[28].

Besides, similar to the Portuguese population[32], the *IL-1B*-511 and *IL-1RN* (VNTR) loci were observed to be in a weak linkage disequilibrium with each other, among controls[28]. Furthermore, on analyzing the effect of *IL-1B* (-511C/T) genotypes on its levels, a substantial decrease in the levels was evident in TT genotyped controls, with respect to those with the heterozygous (CT) genotype but not in HBV-infected individuals. This observation was in line with the previous documentation of an up-regulated IL-1B production due to the presence of C allele[33].

IL-6

This is a 23.7 kDa pleiotropic cytokine, produced by both lymphoid and non-lymphoidcells[34]. This cytokine acts as both pro-as well as anti-inflammatory cytokine and has a key role in growth-promotion and anti-apoptotic activities[35]. The genes involved in processes like differentiation, survival, apoptosis and proliferation are mainly targeted by the IL-6 family[36]. Inter-individual variations at transcription and expression level occur due to IL-6 polymorphisms (promoter region)[37]. Studies conducted so far, have reported three SNPs located in the *IL-6* gene promoter (-597G/A, -572C/G and -174G/C), which result in up-regulation of IL-6 levels and have been observed in chronic hepatitis B patients. The association analysis carried out by us (Table 2), between *IL-6* (-572) genotypes and the HCC risk, showed that in case of GC genotype, a significant negative association was evident for HCC development, among carriers. While the CC genotype, acted as vital protective factor for cirrhosis development[38]. However, a Korean study reported a non-significant association of *IL-6*-572 (G > C) polymorphism with hepatitis outcome, *i.e.*, the occurrence of liver cirrhosis and HCC following hepatitis, in individuals hetero-and homozygotes for G allele, as compared to the CC homozygotes (Table 1)[39]. Further, on associating *IL-6* (-597) genotypes with HCC susceptibility, the heterozygous genotype (GA) was significantly in negative association with HCC risk, among HBV carriers. Besides, when we determined IL-6 haplotypes with the HCC risk, haplotypes 2 (GA) and 3 (CG) were found to be significantly positively associated with HCC development, while the haplotype 4 (CA) acted as a potential protective factor for the same. Additionally, no difference was evident in IL-6 levels in case of *IL-6* (-572) and *IL-6* (-597) genotypes, in our study (Tables 1 and 2)[38]. However, earlier, a study conducted in healthy Spanish population, showed that G allele at -597 is associated with significantly elevated IL-6 circulating levels[40].

IFN-γ

This cytokine has a multifunctional role, produced exclusively by T lymphocytes and natural killer (NK) cells[41]. Several reports have indicated the significance of *IFN-γ* gene polymorphism (+874), situated in its first intron, which coincides with the nuclear factor κB

binding area[41], in modulating HBV infection risk. In our lab, the association analysis conducted between the *IFN-γ* (+874 T > A) genotypes and the cancer risk in Indian population, showed that the heterozygous genotype (TA) was significantly in negative association with hepatitis and later on with HCC development, in healthy controls as well as HBV-inactive carriers. The variant AA genotype was also observed to be in significant negative association with HBV-HCC risk, among controls as reference (Tables 1 and 2)[42]. The results differed from study by Cheong *et al*[43], where no significant association was evident between IFN-γ (+874) polymorphism and susceptibility to HBV infection (Table 1). Studies by several authors carried out in different populations (Colakogullari *et al*[44]; Farhat *et al*[45]; and Forte *et al*[46]), showed that the levels of wild genotype individuals were significantly elevated when compared to TA genotype subjects while, we did not observe such changes in IFN-γ levels among individuals with different genotypes[42].

IL-10

IL-10 is regarded as a pleiotropic Th2 cytokine, mainly involved in regulation of inflammatory responses. It primarily participates in inhibiting cytokine synthesis by Th1 cells[47]. It acts both as an anti-inflammatory (tumorigenic) and anti-angiogenic (anti-tumorigenic) cytokine. Further, as reported by Breen *et al*[48], *IL-10* upstream promoter region has two linked biallelic SNPs at positions -819 (C/T) and -592 (C/A).

Upon studying the association analysis of *IL-10* genotypes with HBV-HCC risk, we found the CC/TA genotype to be in a significant positive association with HBV-HCC development (Tables 1 and 2)[49]. While a study conducted by Nieters *et al*[50], in Chinese population, showed that the wild and heterozygous genotypes shared no significant association with HCC (Table 1). Moreover, the haplotype analysis, revealed a strong linkage disequilibrium between the two studied single nucleotide polymorphisms, consistent to the other studies by Breen *et al*[48]; Shin *et al*[51]; Tseng *et al*[52]; and Gambhir *et al*[53]. However, in case of Indian population, no significant association was found between the 2 haplotypic combinations (CC and TA) observed and HCC risk (Tables 1 and 2)[49]. On the contrary, the CC haplotype was found to accelerate the HCC progression rate in HBV patients in a study by Shin *et al*[51].

IL-12

IL-12, a key Th1 proinflammatory cytokine and is produced chiefly by the antigen presenting cells. This heterodimeric cytokine suppresses the Th2 function and was initially recognized as a connecting link between innate and adaptive immune responses. It's major biological functions include activation of NK and T cells, causing induction of IFN-γ and imparts resistance to tumors, by promoting Th1 adaptive immunity and cytotoxic T lymphocyte responses. Besides, several molecular epidemiologic studies have stated the functional importance of SNP at +1188 (A/C) in the 3' untranslated region (3'UTR) of *IL-12p40*/*IL-12B* in immune mediated diseases and cancer risk.

The association study done between the *IL-12B* (+1188 3'UTR) genotypes and HCC risk, revealed no significant association between the AC and CC genotypes with HCC risk (Tables 1 and 2)[54]. Similar observations were reported in two separate studies done in the Chinese population, where these genotypes of *IL-12B* were not found to be significantly associated with HBV induced HCC (Table 1)[50,55]. Another study done in HCV patients, showed that the association of AC genotype with self-limited infection, while the persistent HCV infection was observed to be associated with AA genotype[56]. The presence of "A" allele at *IL-12B* (+1188 3'UTR) resulted in elevated IL-12B production[57].

TNF-α

It is a potent pleiotropic cytokine. It's gene is located on the short arm of human chromosome 6 (6p21.3)[58]. TNF-α is a proinflammatory and an immunomodulatory cytokine. Various studies have shown that TNF-α, along with IFN-γ exerts an antiviral effect, profoundly suppressing *HBV* gene expression in infected hepatocytes noncytolytically. Literature has shown, several functional SNPs in the TNF-α promoter region, which were reported to influence the TNF-α constituitive and inducible expression levels. Till date, however, the best described SNP is at -308 position of the *TNF-α* promoter.

A study conducted by Jeng *et al*[59], showed that the TNF308.2 (A) allele significantly contributes to a higher HCC risk in Taiwanese population (Table 1). However, in our study in Indian population[42] and in a study by Somi *et al*[60] in Iranian population, no such association was observed (Tables 1 and 2). Numerous studies have observed the TNF2 allele(A) to be a stronger transcriptional activator than wild (G) allele[61-64]. On the contrary, no significant difference was evident between the *TNF-α* (-308) genotypes, its serum and *ex vivo* levels in Chilean rheumatoid arthritis patients[65], Taiwanese[66] and the Asian Indians[67].

TGF-β1

TGF-β, a polypeptide growth factor family, being encoded by three different genes-*TGF-β1*, *TGF-β2,* and *TGF-β3.* Among these, TGF-β1 is most frequently up-regulated in tumor cells[68]. TGF-β1, a multifunctional cytokine, acts a potent growth inhibitor in wound healing and differentiation processes. Owing to this, great stress has been laid on studies about impact of TGF-β1 and its gene variations in susceptibility/pathogenesis of various diseases. So far, many *TGF-β1* polymorphisms have been documented *viz*. three variations, located upstream of exon 1 (at positions -988C/A, -800G/A, and -509C/T), an insertion/deletion of cytosine residue in the 5'UTR (at position +72) and three nucleotide substitutions in the gene's coding region[69]. However, the most reported -509 C > T polymorphism in *TGF-β1*

promoter is linked with its increased circulating levels.

The association analysis concerning the *TGF-β1* (-509) genotypes with HBV-HCC risk, revealed that both hetero- and homozygotes for the T allele, acted as vital risk factors for HCC, in Indian healthy subjects. While, the variant genotype acted as a significant protective factor for cirrhosis and the subsequent HCC risk, among inactive carriers (Tables 1 and 2)[54]. Similarly, a study reported significantly lowered HCC risk in hepatitis B patients with variant (TT) genotype, than in those with wild (CC) genotype[70] and another study also reported decreased HCC risk in patients with TT or CT genotypes than in those with the wild genotype[71]. Both the CC and TT genotypes were found to be significant risk factors for cirrhosis in an earlier study done in Italian population (Table 1)[72]. Besides, the -509C allele was also observed to be significantly associated with higher HCV clearance rates ($P < 0.01$), in a study by Kimura *et al*[73]. A Chinese case-control study revealed that both T allele heteo- and homozygotes were significantly associated with decreased colorectal cancer risk[74].

Grainger *et al*[75], have observed that the T allele of -509C/T polymorphism accounts for higher TGF-β1 production. However, our study differed from this finding as no substantial difference in the levels in any of the *TGF-β1* genotypes was observed[54]. Further, a study done by Qi *et al*[70], also did not show any significant difference in TGF-β1 plasma concentration, between CC and TT genotypes among diseased or healthy controls. Ethnic disparity could be the most probable reason for the apparent discrepancy on the genetic control of TGF-β1 production level.

IL-2

IL-2, a proinflammatory and strong immunoregulatory Th1 cytokine, affecting various immune cells. John *et al*[76] had reported two SNPs in *IL-2* gene (-330 and +166). The +166 change occurs in the leader peptide, so no change occurs in amino acid sequence. The SNP at -330 promoter region position produces two alleles (T and G). Since, the -330 promoter region polymorphism consists of two common alleles, so it is regarded as an appropriate marker for association studies.

On associating the *IL-2* (-330 T > G) genotypes with HCC progression in HBV infected individuals, we showed that both the TG and GG genotypes remained largely non-significant in HBV chronicity, among controls and carriers[54]. Similarly, the *IL-2* (-330 T > G) polymorphism did not appear to modify HBV-HCC risk in the Chinese and American populations[50,77]. On the contrary, a study by Gao *et al*[78] reported, *IL-2*-330 TT genotype to be associated with an increased risk of chronic hepatitis, in case of either HBV or HCV or HBV-HCV coinfection in Chinese population (Table 1).

IL-4

Both IL-4 and IL-10, are cytokines secreted by Th2 cells, and suppress the generation of Th1 response[50]. IL-4, the prime Th2 cytokine, act antagonistically to various IFN-stimulated functions on Th1 differentiation/stability[79]. *In vitro* and *in vivo* studies had documented the T allele of IL-4 (C-590T) polymorphism, which is in linkage disequilibrium with-33T, to be associated with an increased *IL-4* expression.

The association study showed that the CT genotype was found to be potentially negatively associated with hepatitis B development in healthy Indians (Tables 1 and 2)[54]. On the contrary, in a Chinese cohort study, *IL-4* (-590 C > T) genotypes were not found to be significantly associated with the HCC risk in American population (Table 1)[50]. Besides, in another study, *IL-4* (-590) CT and CC genotype frequencies were significantly higher in chronic hepatitis B patients with abnormal ALT levels, thereby associating them with liver inflammatory injury[78]. Moreover, subjects harboring the *IL-4* (-590) CT genotype, showed significantly raised IL-4 levels, with respect to CC genotype subjects (Tables 1 and 2)[54]. Earlier studies have shown enhanced promoter strength with the variant (T) allele at position -590 due to increased binding of the nuclear transcription factors to the promoter, thus up-regulating IL-4 expression.

CONCLUSION

The association studies carried out with cytokine gene polymorphism and HBV related disease chronicity vary considerably across different populations studied. Due to ethnic variability of the results, it is difficult to conclude the associations based on the available data. In nutshell, on the basis of these observations, it can be said that there is a dire need for analyzing the individual and collective polymorphic forms of various cytokines, both mRNA and the protein expression, the correlation between them, in a larger set of individuals in various set of populations, so as to enhance, not only the diagnostic and prognostic value of such studies, but also for determining an individual's susceptibility to HBV-HCC disease.

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MINIREVIEWS

Autoantibodies against tumor-associated antigens for detection of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common tumors worldwide. The survival rate after the onset of symptoms is generally less than one year for the late presentation of HCC, and reliable tools for early diagnosis are lacking. Therefore, novel biomarkers for the early detection of HCC are urgently required. Recent studies show that the abnormal release of proteins by tumor cells can elicit humoral immune responses to self-antigens called tumor-associated antigens (TAAs). The corresponding autoantibodies can be detected before the clinical diagnosis of cancer. Therefore, there is growing interest in using serum autoantibodies as cancer biomarkers. In this review, we focus on the advances in research on autoantibodies against TAAs as serum biomarker for detection of HCC, the mechanism of the production of TAAs, and the association of autoantibodies with patients' clinical characteristics.

Key words: Hepatocellular carcinoma; Diagnosis; Serological marker; Autoantibody; Tumor associated antigen

Core tip: There is growing interest in using serum autoantibodies as cancer biomarkers. However, the mechanism and clinical association of autoantibodies in hepatocellular carcinoma (HCC) remains unclear. In this review, we focus on the advances in research on autoantibodies against tumor-associated antigens (TAAs) as serum biomarker for detection of HCC, the mechanism of the production of TAAs, and the association of autoantibodies with patients' clinical characteristics.

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INTRODUCTION

Liver cancer is the sixth most common malignant disease worldwide, and approximately 50.5% of new cases and 51.4% of cancer-related deaths occur in China[1]. The survival rate after the onset of symptoms is generally less than one year for the late presentation of hepatocellular carcinoma (HCC), and reliable tools for early diagnosis are lacking.

Ultrasound is recommended as a screening tool for early detection of HCC, although it is not very sensitive and is highly operator dependent. Computed tomography is not recommended as a screening tool for HCC because of radiation exposure[2,3]. One current focus of HCC research is the development of a blood test to aid in the diagnosis of this disease. Many serologic biomarkers of HCC are available, including alpha-fetoprotein (AFP), des-γ-carboxyprothrombin, lens culinaris agglutinin-reactive AFP-L3[4,5], Dickkopf-1[6], and squamous cell carcinoma antigen[7]. To date, AFP is the only serum biomarker available for HCC surveillance. However, AFP does not yield satisfactory results to diagnosis HCC in its early stages. Specifically, using a cutoff value of 20 ng/mL, the sensitivity and specificity of AFP assays range between 41%-65% and 80%-90%, respectively, and the sensitivity is lower when AFP is used to detect early-stage HCC[8]. Therefore, novel serum biomarkers that detect HCC before symptoms are apparent are urgently required.

The immune system is the first line of defense against pathogens. During the earliest stage of tumorigenesis, proteins released by tumor cells, or peptides at the surface of tumor cells, can elicit humoral immune responses against the tumor and are therefore called tumor-associated antigens (TAAs). The production of TAAs is not completely understood. The proteins are likely mutated, overexpressed, posttranslationally modified, misfolded, aberrantly cleaved, or aberrantly localized in tumor cells[9]. Therefore, autoantibodies against TAAs are readily isolated, because they are secreted, and their titers increase in response to robust biological amplification of TAAs. Most important, TAAs can be detected before the clinical diagnosis of cancer. Further, unlike polypeptides, antibodies are highly stable in serum and are not proteolyzed. The half-life of TAAs in the bloodstream ranges between 7-30 d, depending on the subclass of immunoglobulin, and may persist for as long as the immunizing autoantigen, which simplifies sample preparation[10]. Thus, detection of anti-TAA autoantibodies will be easier than detecting TAAs themselves, suggesting that the measurement of anti-TAA antibodies may offer the potential to improve upon assays employing conventional biomarkers[11]. For example, Li *et al*[12] reported that the elevated levels of serum antibodies against insulin-like growth factor-binding protein-2 allowed detection of early-stage cancers.

In this review, we discuss advances in research on autoantibodies against TAAs as biomarkers for the detection of HCC, with particular focus on the mechanism of the production of TAAs and the association of autoantibodies with clinical parameters.

ANTI-TAA AUTOANTIBODIES IDENTIFIED IN PATIENTS WITH HCC

The number of reports of TAAs in patients with HCC has recently increased. The main TAAs reported since 1993 are listed in Table 1. Among them, the tumor suppressor protein p53 is one of the most highly immunogenic TAAs identified to date. The prevalence of serum anti-p53 antibodies among HCC patients ranges from 12.2%-73.07%[13-15]. The reasons for the differences are unknown but may be caused by unidentified biological and geographical differences in study populations. Except for HCC, antibodies against p53 are present in patients with many types of cancer and may provide a tool for detection of cancer recurrence[16].

The insulin-like growth factor mRNA-binding (IMP) family member IMP2 binds to mRNA and regulates translation of the mRNA that encodes insulin-like growth factor 2 and is frequently reported as a TAA in patients with HCC[17-19]. Members of the IMP family are oncofetal proteins, which disappear from all tissues soon after birth but are frequently re-expressed during the malignant transformation of numerous cell types. IMP2 was first identified as a TAA for HCC in 1999[20]. An autoantibody against IMP2 is present in 21% of patients with HCC patients, but is undetectable in precursors such as chronic hepatitis and liver cirrhosis[20].

Elevated levels of autoantibodies to calreticulin[21], cyclin B1[22], centromere protein F (CENPF)[23], and survivin[24,25] are frequently detected in the sera of patients with HCC. However, no autoantibody binds its immunogen with enough sensitivity to detect HCC[26]. To overcome this drawback, multi-autoantibody panels were applied to improve sensitivity. For example, Zhang and colleagues[27] constructed an antigen microarray comprising IMP1, IMP2, IMP3, p53, c-myc, cyclin B1, survivin, and p16, and the results show that the frequency of antibody detection to any individual TAA of patients with HCC varied from 9.9%-21.8%. With the successive addition of TAAs of all eight antigens, there was a stepwise increase in positive antibody reactions, reaching a frequency of 59.8% in an entire cohort. This shows that a mini-array of eight TAAs enhanced antibody detection for the diagnosis of HCC. When Sui1 and RalA were added to the panel, the final cumulative prevalence of anti-TAA antibodies increased to 66.2% (51/77)[28]. Therefore, multi-autoantibody panels might

Table 1 Tumor-associated antigens detected in patients with hepatocellular carcinoma reported since 1993

Ref.	TAAs reported
Yau *et al*[36]	hnRNP L
Akada *et al*[42]	HSP70, SOD2, and PRDX6
Shao *et al*[41]	Glucose-regulated protein 78
Nomura *et al*[39]	Ku86
Liu *et al*[40]	CENPF, DDX3, HSPA4, HSPA5, VIM, LMNB1, and p53
Pekáriková *et al*[21]	CRT
Chen *et al*[28]	Sui1, RalA
Wang *et al*[43]	KRT23, AHSG and FTL
Wang *et al*[44]	RalA
Looi *et al*[45]	HSP60, HSP70
Li *et al*[35]	DDX3, eEF2, AIF, hnRNP A2, PBP, and TIM
Chen *et al*[46]	EIF3SI, LDHA, RFC2, and MCART1
Zhang *et al*[27]	IMP1, IMP2, IMP3, p53, c-myc, cyclin B1, survivin and p16
Akere *et al*[13]	p53
Zhou *et al*[47]	HCC-22-5
Takashima *et al*[48]	HSP70, GAPDH, PRX, Mn-SOD
Looi *et al*[49]	p16
Yagihashi *et al*[25]	Survivin
Su *et al*[17]	IMP2
Himoto *et al*[19]	IMPs
Himoto *et al*[18]	IMPs, p53, c-myc, and survivin
Zhang *et al*[24]	c-myc, cyclin B1, IMP1, Koc, p53, IMP2, and survivin
Soo Hoo *et al*[50]	p53, IMP2, Koc, CENP-F, p90
Le Naour *et al*[51]	CRT, CK8, NDK-A, and ATP5B
Zhang *et al*[23]	IMP2, CENPF
Zhang *et al*[20]	IMP2
Raedle *et al*[52]	p53
Covini *et al*[22]	Cyclin B1
Imai *et al*[53]	HCC1

TAAs: Tumor-associated antigens; HCC: Hepatocellular carcinoma; IMP: Insulin-like growth factor mRNA-binding; CENPF: Centromere protein F.

be useful tools for HCC diagnosis.

A feature of HCC is that antecedent liver cirrhosis and chronic hepatitis are common precursors, and 80%-90% of patients with cirrhosis develop HCC[29]. Autoantibodies to TAAs are detected during the transition to malignancy[30]. It was proposed that these antibody responses might be stimulated by cellular proteins that are involved in carcinogenesis. Thus, cirrhosis-associated autoantibodies can identify individuals at risk of developing HCC.

MECHANISM OF THE PRODUCTION OF AUTOANTIBODIES AGAINST TAAs

The mechanism of generation of autoantibodies against TAAs is not fully understood. TAA proteins are likely either mutated, overexpressed, or aberrantly localized in tumor cells[9]. Autoantibodies may be elicited by proteins with incorrect posttranslational modifications that are recognized as nonautologous[31]. Phosphorylation, glycosylation, oxidation, or proteolytic cleavage may generate a neo-epitope with affinity for the major histocompatibility complex or T-cell receptor that induces an immune response[32]. For example, HSP60 localizes mainly to mitochondria, but in tumor cells it is present in the cytoplasm and cell membrane, leading to the induction of an autoimmune response[33]. Moreover, the level of expression of HSP60 is significantly higher in breast tumor tissues, suggesting that overexpression of HSP60 may represent a mechanism of developing immunogenicity in patients with breast cancer[33]. Similarly, our recent study shows that the high titer of anti-CENPF autoantibody in HCC serum is likely caused by an autoimmune reaction in response to overexpression of CENPF[34].

ASSOCIATION OF THE PREVALENCE OF AUTOANTIBODIES WITH THE CLINICAL CHARACTERISTICS OF PATIENTS WITH HCC

There are relatively few studies on the clinical significance of autoantibodies in patients with HCC because of insufficient numbers of patients and the lack of accurate clinical information. There is evidence, however, showing that there are no statistically significant differences in patients with HCC in the prevalence of autoantibodies against DEAD box 3, eEF2, AIF, hnRNP A2, PBP, and TIM and patients' characteristics of sex, histological grade, or TNM classification[35]. However, tumors > 5 cm in diameter are present more frequently in patients with anti-eEF2 autoantibodies compared with those with small tumors (> 5 cm in diameter) ($P < 0.05$)[35]. The rates of detection of autoantibodies against AIF and hnRNP A2 in patients with HCC without regional lymph node metastasis were significantly higher compared with those with regional lymph node metastasis ($P < 0.05$)[35]. There is a significant difference in size of tumors of patients with HCC cases that correlates with prevalence of autoantibodies against hnRNP L-67-88, with the average tumor size of 5.84 ± 4.23 cm in patients with detectable autoantibodies whereas 3.70 ± 2.07 cm in patients without detectable autoantibodies[36]. Survival analysis shows that the survival rates of patients with hepatitis B virus-positive HCC with autoantibodies are significantly lower compared with those without detectable autoantibodies ($P < 0.05$), indicating that an elevated level of autoantibody against hnRNP L-67-88 is associated with larger tumors and poorer prognosis[36].

In our recent study (data not shown), analysis of clinicopathological associations shows that the prevalence of positive for autoantibodies against CENPF and HSP60 is higher in patients with HCC < 50 years of age. The prevalence of autoantibodies against CENPF is higher in patients with well-differentiated HCC with Child-Pugh grade A liver function. In contrast, there are no data available, to our knowledge that associates autoantibodies against p53 with patients' clinical characteristics. In patients with colorectal cancer (CRC), there is an increase in the prevalence of anti-p53 autoantibodies in carcinoma *in-situ* (6%) compared with adenomas (1%), indicating that the level of anti-p53 autoantibody increases with CRC

progression[37]. However, almost all studies report that there is no association between anti-p53 autoantibodies and CRC stage progression[37], and only a handful of studies suggest an association between anti-p53 autoantibody and T-stage, selected nodal disease, and metastases[37], suggesting that the autoantibody may have more value in the early diagnosis of cancer than for prognosis. However, subanalysis of autoantibody detection rates in tumors of different causes or stage was not possible in many studies, because of unknown cause or lack of tumor-stage data of many of the HCC samples[38].

PROSPECTS

During the past few years, the potential utility of autoantibodies against TAAs as biomarkers for HCC has been explored. However, their value for this purpose is controversial. There is concern that there is no single anti-TAA autoantibody with high sensitivity and specificity that detects HCC, and no large-scale clinical trial has been conducted to validate candidate TAAs[26,28,35,39-41]. Further studies of large populations with precise clinical information should be conducted to determine whether autoantibodies to TAAs are associated with patients' clinical characteristics as well studies on the mechanism of the production of TAAs, with the aim of clarifying the role of specific TAAs as biomarkers for the early diagnosis and prognosis of HCC.

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ORIGINAL ARTICLE

Prospective Study

Burden of nonalcoholic fatty liver disease and advanced fibrosis in a Texas Hispanic community cohort

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding authors at jenjung.pan@uth.tmc.edu.

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Abstract

AIM: To investigate the potential burden of nonalcoholic steatohepatitis (NASH) and advanced fibrosis in a hispanic community.

METHODS: Four hundred and forty two participants with available ultrasonography data from the Cameron County Hispanic Cohort were included in this study. Each participant completed a comprehensive questionnaire regarding basic demographic information, medical history, medication use, and social and family history including alcohol use. Values of the nonalcoholic fatty liver disease fibrosis score (NFS), FIB4 index, BARD score, and Aspartate aminotransferase to Platelet Ratio Index (APRI) were computed using the blood samples collected within 6 mo of liver ultrasonography from each participant. Hepatic steatosis was determined by ultrasonography. As part of univariable analysis, for continuous variables, comparisons among groups were performed with student-*t* test, one way analysis of variance, and Mann-Whitney test. Pearson χ^2 and the Fisher exact test are used to assess differences in categorical variables. For multivariable analyses, logistic regression analyses were performed to identify characteristics associated with hepatic steatosis. All reported *P* values are based two-sided tests, and a *P* value of less than 0.05 was considered to indicate statistical significance.

RESULTS: The mean age and body mass index (BMI) of the study participants were 49.1 years and 31.3 kg/m^2, respectively. Among them, 65.6% were females, 52% had hepatic steatosis, 49.5% had metabolic syndrome, and 29% had elevated aminotransferases. Based on established cut-offs for diagnostic panels, between 17%-63% of the entire cohort was predicted to have NASH with indeterminate or advanced fibrosis. Participants with hepatic steatosis had significantly higher BMI (32.9 ± 5.6 kg/m^2 *vs* 29.6 ± 6.1 kg/m^2, $P < 0.001$) and higher prevalence rates of elevation of ALT (42.2% *vs* 14.6%, $P < 0.001$), elevation of aspartate aminotransferase (38.7% *vs* 18.9%, $P < 0.001$), and metabolic syndrome (64.8% *vs* 33%, $P < 0.001$) than those without hepatic steatosis. The NFS scores ($P = 0.002$) and the APRI scores ($P = 0.002$) were significantly higher in those with steatosis but the scores of the FIB4 index and BARD were similar between the two groups. After adjusting for age, gender and BMI, elevated transaminases, metabolic syndrome and its components, intermediate NFS and APRI scores were associated hepatic steatosis in multivariable analysis.

CONCLUSION: The burden of NASH and advanced fibrosis in the Hispanic community in South Texas may be more substantial than predicted from referral clinic studies.

Key words: Noninvasive biomarkers; Nonalcoholic fatty liver disease; Hispanics; Ultrasonography; Liver fibrosis

Core tip: Among different racial and ethnic populations in the United States, Hispanics (predominantly of Mexican origin) are at particular risk for nonalcoholic fatty liver disease (NAFLD) and appear to have a more aggressive disease course. From the risk stratification and early intervention perspective, it is pivotal to define the magnitude of the burden of NAFLD in asymptomatic individuals in Hispanic communities and identify the subset with nonalcoholic steatohepatitis (NASH). Such community based data are scarce. In this study, we assessed the potential burden of NASH and advanced fibrosis in a Hispanic community utilizing four common diagnostic panels and ultrasonography.

Pan JJ, Fisher-Hoch SP, Chen C, Feldstein AE, McCormick JB, Rahbar MH, Beretta L, Fallon MB. Burden of nonalcoholic fatty liver disease and advanced fibrosis in a Texas Hispanic community cohort. *World J Hepatol* 2015; 7(11): 1586-1594 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i11/1586.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i11.1586

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of liver injury ranging from simple steatosis with a more benign course to nonalcoholic steatohepatitis (NASH) which may progress to advanced fibrosis, cirrhosis and liver cancer[1,2]. Among different racial and ethnic populations in the United States, Hispanics (predominantly of Mexican origin) are at particular risk for NAFLD and appear to have a more aggressive disease course[3]. Hispanics accounted for nearly 50% of the United States population growth from 2000 to 2010 and are projected to reach 30% of the United States population within the next three decades[4]. Given the increasing prevalence and the expected growth in the Hispanic population, NAFLD poses a significant threat to this population.

From the risk stratification and early intervention perspective, it is pivotal to define the magnitude of the burden of NAFLD in asymptomatic individuals in Hispanic communities and identify the subset with NASH. Such community based data are scarce. Liver biopsy remains the standard for the diagnosis and staging of NASH, although invasiveness and cost preclude its use as a screening tool in general populations[5]. Markers that either individually or as composite panels predict the presence of NASH and advanced fibrosis of liver have been developed[6]. These panels have been advocated as a means to target liver biopsy to those at increased risk by identifying those at low, intermediate or high risk of NASH and advanced fibrosis. Practice guidelines recommend the NAFLD fibrosis score (NFS) as a clinically useful tool for identifying patients at higher likelihood

of having bridging fibrosis and/or NASH[7]. Moreover, three panels derived from common anthropometric, hematological and biochemical parameters, the FIB4 index, the BARD score, and Aspartate aminotransferase (AST) to Platelet Ratio Index (APRI) are used to predict advanced fibrosis in NAFLD[8-10]. These biomarkers and panels have been validated in cross-sectional studies of non-Hispanic cohorts evaluated for liver abnormalities but have not been evaluated in a population setting in Hispanics.

In this study, we assessed four diagnostic panels for NASH and fibrosis in relation to demographic, laboratory and liver ultrasonography data in asymptomatic individuals enrolled in the Cameron County Hispanic Cohort (CCHC); an extensively studied ethnic population-based cohort of community-dwelling Mexican Americans followed longitudinally who live in a city on the Texas-Mexico border.

MATERIALS AND METHODS

Study participants

This study has been approved by the institutional review board of the University of Texas Health Science Center at Houston. Written consent was obtained from each participant. The CCHC (n = 3200) was originally established in 2003 and the participants were randomly selected based on the 2000 Census tract data in the city of Brownsville, Texas. Over 90% of them are Mexican Americans[11]. During initial visit, the participants completed a comprehensive questionnaire regarding basic demographic information, medical history, medication use, and social and family history including alcohol use. Starting in 2012, consenting participants were offered liver ultrasonography performed prospectively for the assessment of hepatic steatosis. The scores of the diagnostic panel for each participant were retrospectively computed using the blood samples collected within 6 mo of liver ultrasonography. Blood samples were taken and plasma aliquots immediately stored at -70 ℃ for a range of assays. Plasma glucose and complete blood count were performed on site. Stored specimens were sent in batches to a clinical laboratory for biochemistries, including hepatic function tests. Hepatitis C antibody was measured using the ORTHO® hepatitis C virus Version 3.0 Elisa Test System (Ortho Clinical Diagnostics Inc, Rochester, NY).

Liver ultrasonography

Ultrasonography was performed using an established protocol[12]. Study participants were asked to be fasting for at least 6 h prior to ultrasound examination to maximize the distention of gall bladder and to reduce food residue and gas in the upper gastrointestinal tract which may reduce image quality or preclude liver imaging. Trained technicians performed the abdominal ultrasonography with a 5 MHz transducer (Ch5-2, Siemens, Mountain View, CA, United States). During the scan, liver parenchyma was examined subcostally and intercostally in the decubitus position as well in modified slightly oblique positions with the right arm above the head and the right leg stretched during all respiration cycles to identify the best approach and to avoid artefacts caused by the thorax. For diagnosis of hepatic steatosis, the following features were recorded: (1) Ultrasonographic contrast between the liver and right renal parenchyma of right intercostal sonogram in midaxillary line; (2) Brightness of the liver; (3) Deep attenuation of echo penetration into the deep portion of the liver and impaired visualization of the diaphragm; and (4) Impaired visualization of the borders of intrahepatic vessels and narrowing the their lumen. The overall gain, initial gain, and time gain compensation settings were kept within a narrow range. The ultrasonographic images were interpreted by one person (JJP) in a blinded fashion.

Definitions

The presence of hepatic steatosis is qualitatively defined as a brighter liver parenchyma than the right kidney on ultrasonography. Based on the third National Health and Nutrition Examination Survey (NHAHES Ⅲ 1988-1994) definition, abnormal aminotransferases are defined as alanine aminotransferase (ALT) greater than 40 U/L for men and greater than 31 U/L for women; AST greater than 37 U/L for men and greater than 31 U/L for women. According to the United States National Cholesterol Education Program Adult Treatment Panel Ⅲ, the metabolic syndrome (MetS) is defined as the presence of at least 3 of the following 5 components: elevated waist circumference (> 102 cm for men and > 88 cm for women), elevated triglycerides (≥ 150 mg/dL), reduced high-density lipoprotein cholesterol (HDL-C) (< 40 mg/dL for men and < 50 mg/dL for women), elevated blood pressure (≥ 130/85 mm Hg or use of medication for hypertension), and elevated fasting glucose (≥ 110 mg/dL)[13]. The formulae and cut-off scores of the diagnostic panels for detection of liver fibrosis are shown in the Table 1.

Statistical analysis

Descriptive data are presented as either means ± SD or median (interquartile range) for continuous variables, depending on whether the distribution of the variables is symmetrical or skewed. Frequencies and percentages are reported for categorical variables. As part of univariable analysis, for continuous variables, comparisons among groups were performed with student-t test, one way analysis of variance, and Mann-Whitney test. Pearson χ^2 and the Fisher exact test are used to assess differences in categorical variables. For multivariable analyses, we used logistic regression. All reported P values are based two-sided tests, and a P value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed with SPSS 22.0 (SPSS Inc., Chicago, IL). A statistical review of the study was performed by a biomedical statistician (Dr. Mohammad H Rahbar).

Table 1 Formulae and cut-off scores of diagnostic panels for detection of liver fibrosis

Diagnostic panel	Formula	Cut-off scores for advanced fibrosis	
		Absent	Present
BARD score[8]	Scale 0-4 BMI ⩾ 28 kg/m^2 = 1 point AST/ALT ratio ⩾ 0.8 = 2 points DM = 1 point		⩾ 2
FIB4 index[9]	Age (yr) × AST (U/L)]/(platelet (10^9) × [ALT (U/L)]$^{1/2}$	< 1.3	> 2.67
APRI[10]	(AST/ULN)/platelets (10^9/L) × 100	⩽ 0.5	> 1.5
NAFLD fibrosis score[15]	-1.675 + 0.037 × age (yr) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/DM (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet (× 10^9/L) - 0.66 × albumin (g/dL)	< -1.455	> 0.676

BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ULN: Upper limit of normal; DM: Diabetes mellitus; IFG: Impaired fasting glucose; NAFLD: Nonalcoholic fatty liver disease.

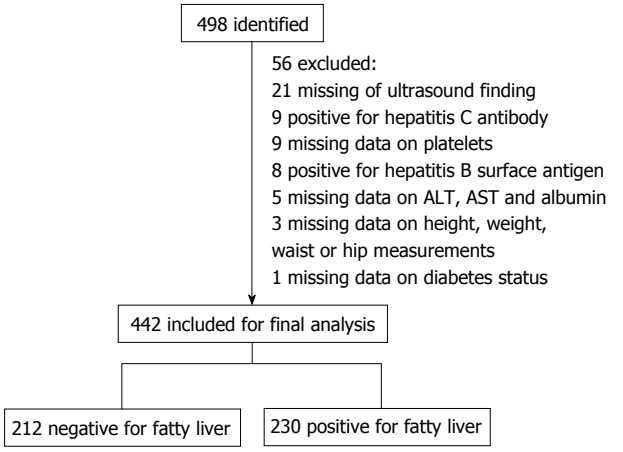

Figure 1 Overview of the study. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

RESULTS

Characteristics of the study participants

Four hundred and forty two participants were included in this study. Figure 1 shows an overview of the inclusion of individuals. Among the 498 consecutive participants recruited to this study, 56 were excluded for the following reasons: lack of data on liver ultrasonography (*n* = 21), positive Hepatitis C antibody (*n* = 9), lack of data on platelet counts (*n* = 9), positive Hepatitis B surface antigen (*n* = 8), lack of data on ALT, AST or albumin levels (*n* = 5), lack of data on height, weight, waist or hip measurements (*n* = 3), and lack of data on diabetes status (*n* = 1). Among the 442 remaining participants in this study, none reported excessive alcohol use (< 20 g of alcohol/day). As shown in Table 2, the mean age and body mass index (BMI) of the study participants was 49.1 years and 31.3 kg/m^2, respectively. Among the participants in this study, 65.6% were females, 52% had hepatic steatosis, and nearly half had MetS. Approximately one-third of the participants had elevated ALT and AST. Ten participants had serum albumin less than 3.5 g/dL and 11 had platelet counts less than 150 × 10^9/μL. None of the participants with either hypoalbuminemia or thrombocytopenia had ascites on ultrasound. Regarding the individual components of the MetS, 37.8% had hypertension, 30.3% had hyperglycemia, 39.6% had hypertriglyceridemia, 73.3% had central obesity, and 60.4% had low HDL-C.

Among the CCHC participants from whom this sample was drawn (*n* = 1847), we previously reported that 67% were females, 39% had elevated ALT (⩾ 40 U/L), and 44% had MetS[14]. Given the similarities between the current report and the previous report, we believe that the study cohort is representative of its parent cohort.

NASH/fibrosis panels in the study participants

Table 2 shows the components and scoring for the diagnostic panels measured in the study. As shown in Table 2, a significant percentage of participants had elevated values on diagnostic panels suggesting the presence of NASH with fibrosis. The median (interquartile range) NFS value in the cohort was -1.63 (-2.57, -0.62). Based on published cut-off scores for the NFS[15], advanced liver fibrosis would be excluded in 55%, present in 7%, and indeterminate in 38% of the study participants. The median (interquartile range) FIB4 index in this cohort was 0.89 (0.58, 1.35). Similarly, based on published cut-off scores[9], advanced liver fibrosis would be excluded in 73%, present in 3% and indeterminate in 24% of the participants. The median (interquartile range) of the BARD score was 2 (1, 3). Using established cut-offs[8], 63% of the cohort would have been predicted to have advanced liver fibrosis. Finally, the median (interquartile range) of the APRI was 0.31 (0.21, 0.45). Using published cut-off values[10], advanced fibrosis would be excluded in 83%, present in 1% and indeterminate in 16% of the cohort. Based on these scores only 26% would have no predicted fibrosis and 0.4% would have predicted advanced fibrosis using all 4 panels. The majority of the cohort (73%) was predicted to have indeterminate risk for advanced liver fibrosis when using all 4 panels.

NASH/fibrosis panels and characteristics of participants with and without hepatic steatosis by ultrasonography

A comparison of two groups of study participants

Table 2 Summary of the characteristics of participants (*n* = 442)

Characteristics	
Age (yr)	49.1 ± 14.2
Gender	
Female	290 (65.6)
Male	152 (34.4)
BMI (kg/m^2)	31.3 ± 6.1
Hepatic steatosis	230 (52.0)
Elevated ALT[1]	128 (29.0)
Elevated AST[2]	129 (29.2)
Albumin (g/dL)	4.0 (3.8-4.2)
Platelet (10^9/μL)	243.5 (207.0-282.0)
DM[3]	97 (21.9)
Hypertension	167 (37.8)
Hyperglycemia	134 (30.3)
Hypertriglyceridemia	175 (39.6)
Low HDL-C	267 (60.4)
Central obesity	324 (73.3)
Metabolic syndrome	219 (49.5)
NAFLD fibrosis score	
< -1.455	244 (55.2)
-1.455-0.676	169 (38.2)
> 0.676	29 (6.6)
FIB4 index	
⩽ 1.31	323 (73.1)
1.31-2.66	106 (24.0)
⩾ 2.67	13 (2.9)
BARD	
0-1	163 (36.9)
2-4	279 (63.1)
APRI	
⩽ 0.5	365 (82.6)
0.51-1.5	73 (16.5)
> 1.5	4 (0.9)

Age and BMI are described as mean ± SD. Other continuous variables are described as median (interquartile range). Categorical variables are described as frequency (%). [1]ALT > 40 U/L for men and > 31 U/L for women; [2]AST > 37 U/L for men and > 31 U/L for women; [3]Either history of diabetes mellitus, use medication for diabetes mellitus, fasting plasma glucose ⩾ 126 mg/dL, or glycosylated hemoglobin A1C ⩾ 6.5 g/dL. BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DM: Diabetes mellitus; HDL-C: High-density lipoprotein cholesterol; APRI: AST to platelet ratio index; NAFLD: Nonalcoholic fatty liver disease.

based on the presence or absence of hepatic steatosis on ultrasonography revealed the following results. As shown in Table 3, there was no difference in either age or gender between the two groups. However, the hepatic steatosis group had significantly higher BMI (32.9 ± 5.6 kg/m^2 *vs* 29.6 ± 6.1 kg/m^2, $P < 0.001$) and higher prevalence rates of elevation of ALT (42.2% *vs* 14.6%, $P < 0.001$), elevation of AST (38.7% *vs* 18.9%, $P < 0.001$), and MetS (64.8% *vs* 33%, $P < 0.001$) than those without hepatic steatosis. In addition, features of MetS including central obesity (86.5% *vs* 59%, $P < 0.001$), hyperglycemia (41.7% *vs* 17.9%, $P < 0.001$), hypertriglyceridemia (47% *vs* 31.6%, $P = 0.001$), and lower HDL-C levels (70.4% *vs* 49.5%, $P < 0.001$) were significantly more common in participants with hepatic steatosis than those without hepatic steatosis. The NFS scores ($P = 0.002$) and the APRI scores ($P = 0.002$) were significantly higher in those with steatosis but the scores of the FIB4 index and BARD were similar between the two groups.

In univariable analysis, BMI [odds ratio (OR) = 1.11; 95%CI: 1.07-1.15, $P < 0.0001$], elevated ALT (OR = 4.26; 95%CI: 2.68-6.76, $P < 0.0001$), elevated AST (OR = 2.71; 95%CI: 1.76-4.19, $P < 0.0001$), MetS (OR= 3.73; 95%CI: 2.52-5.53, $P < 0.0001$) and its components including hyperglycemia (OR = 3.28; 95%CI: 2.12-5.08, $P < 0.0001$), hypertriglyceridemia (OR = 1.92; 95%CI: 1.30-2.83, $P = 0.001$), low HDL-C (OR = 2.43; 95%CI: 1.64-3.59, $P < 0.0001$), and central obesity (OR = 4.47; 95%CI: 2.80-7.13, $P < 0.0001$) were associated with fatty liver on ultrasound. Intermediate (OR = 2.05; 95%CI: 1.37-3.06, $P = 0.0005$) but not high NFS scores (OR = 1.14; 95%CI: 0.53-2.46, $P = 0.74$) were associated with ultrasonographic fatty liver. Similarly, intermediate (OR = 2.52; 95%CI: 1.47-4.32, $P = 0.0008$) were also associated with hepatic steatosis. Neither the FIB4 index nor the BARD was associated with fatty liver on ultrasound (Table 4).

After adjusting for age, gender and BMI, elevated ALT (OR = 4.04; 95%CI: 2.50-6.52, $P < 0.0001$), elevated AST (OR = 2.63; 95%CI: 1.67-4.13, $P < 0.0001$), MetS (OR = 3.33; 95%CI: 2.07-5.01, $P < 0.0001$) and its components including hyperglycemia (OR = 2.87; 95%CI: 1.78-4.61, $P < 0.0001$), hypertriglyceridemia (OR = 1.82; 95%CI: 1.21-2.75, $P = 0.004$), low HDL-C (OR = 1.93; 95%CI: 1.28-2.93, $P = 0.002$) and central obesity (OR = 3.52; 95%CI: 1.94-6.39, $P < 0.0001$) remained as independent factors associated with hepatic steatosis in multivariable analysis. Similarly, intermediate but not high NFS scores (OR= 1.83; 95%CI: 1.10-3.04, $P = 0.02$) and APRI scores (OR = 2.62; 95%CI: 1.49-4.60, $P = 0.0008$) continued to be significantly associated with hepatic steatosis in multivariable analysis. Furthermore, serum albumin level became associated with hepatic steatosis in multivariable analysis (OR = 2.29; 95%CI: 1.07-4.88, $P = 0.03$) (Table 4).

DISCUSSION

This study is unique in examining community-recruited individuals from a population with multiple risk factors for NASH. The presumptive prevalence of liver fibrosis is startling particularly since our sampling methods avoided the bias of studies from specialized clinics. The potential disease burden of advanced liver disease in these Mexican Americans is of great public health concern.

To reach these conclusions we assessed the potential burden of NASH and advanced fibrosis in our participants utilizing four noninvasive diagnostic panels. We found that 52% of all individuals had evidence of hepatic steatosis and thereby NAFLD, in line with the high prevalence of elevated BMI, abnormal aminotransferase levels and MetS in the cohort. Based on established cut-offs for the diagnostic panels, we found

Table 3 Comparison of the characteristics of participants with and without hepatic steatosis

Characteristics	Hepatic steatosis		*P* value
	Absent (*n* = 212)	Present (*n* = 230)	
Age (yr)	49.5 ± 16.3	48.7 ± 12.0	0.6
Gender			
Female	134 (63.2)	156 (67.8)	
Male	78 (36.8)	74 (32.2)	0.3
BMI (kg/m^2)	29.6 ± 6.1	32.9 ± 5.6	< 0.001
Elevated ALT[1]	31 (14.6)	97 (42.2)	< 0.001
Elevated AST[2]	40 (18.9)	89 (38.7)	< 0.001
Albumin (g/dL)	4.0 (3.8-4.2)	4.0 (3.9-4.2)	0.4
Platelets (10^9/μL)	237.5 (204.0-277.8)	251.0 (214.0-283.5)	0.1
DM[3]	29 (13.7)	68 (29.6)	< 0.001
Hypertension	72 (34.0)	95 (41.3)	0.1
Hyperglycemia	38 (17.9)	96 (41.7)	< 0.001
Hypertriglyceridemia	67 (31.6)	108 (47.0)	0.001
Low HDL-C	105 (49.5)	162 (70.4)	< 0.001
Central obesity	125 (59.0)	199 (86.5)	< 0.001
Metabolic syndrome	70 (33.0)	149 (64.8)	< 0.001
NAFLD Fibrosis Score			
< -1.455	134 (63.2)	110 (47.8)	
-1.455-0.676	63 (29.7)	106 (46.1)	
> 0.676	15 (7.1)	14 (6.1)	0.002
FIB4 index			
⩽ 1.3	157 (74.1)	166 (72.2)	
1.31-2.66	47 (22.2)	59 (25.7)	
⩾ 2.67	8 (3.8)	5 (2.2)	0.46
BARD			
0-1	79 (37.3)	84 (36.5)	
2-4	133 (62.7)	146 (63.5)	0.87
APRI			
⩽ 0.5	190 (89.6)	175 (76.1)	
0.51-1.5	22 (10.2)	51 (22.2)	
> 1.5	0 (0)	4 (1.7)	0.002[4]

Age and BMI are described as mean ± SD. Categorical variables are described as frequency (%). [1]ALT > 40 U/L for men and > 31 U/L for women; [2]AST > 37 U/L for men and > 31 U/L for women; [3]Either history of diabetes mellitus, use medication for diabetes mellitus, fasting plasma glucose ⩾ 126 mg/dL, or glycosylated hemoglobin A1C ⩾ 6.5 g/dL; [4]χ^2 with Yate's correction is computed. BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DM: Diabetes mellitus; HDL-C: High-density lipoprotein cholesterol; APRI: AST to platelet ratio index; NAFLD: Nonalcoholic fatty liver disease.

that between 17%-63% of the cohort would be predicted to have NASH with indeterminate or advanced fibrosis, depending on the method used. Evidence of the potential burden of hepatic disease was seen both in those with and without steatosis on ultrasound. However, the severity of the NFS and APRI scores were higher in those with steatosis. These findings, although imprecise, support the hypothesis that the burden of NAFLD related diseases in Mexican Americans on the south Texas border may be even greater than anticipated from published studies. Our data underscore the need to directly assess hepatic histology from the high risk participants we identified in order to validate more precise non-invasive diagnostic panels and to determine the need and targets for therapeutic interventions and prevention.

Our observation that 52% of a population-based Mexican American cohort has NAFLD by ultrasound extends our prior work and substantiates studies where Hispanic individuals were recruited from primary care and gastroenterology clinics from military facilities[16]. This burden of disease parallels the high prevalence of obesity, diabetes and lipid abnormalities in these populations. We may in fact have underestimated the true overall prevalence of advancing liver disease since we were limited in this study to ultrasonography which is not the most sensitive means of detecting liver fat[5]. This is likely, since, many of the individuals who did not have steatosis on ultrasound did have multiple risk factors for NAFLD. It is likely that lesser degrees of steatosis were not appreciated. The fact that our individuals represent a health disparity cohort with diminished access to health care magnifies the potential clinical consequences of NAFLD. This is a population that has little access to therapy for underlying conditions that contribute to liver fat and inflammation.

We were constrained to use a noninvasive approach to explore the potential burden of NASH and advanced fibrosis in this cohort, since at this stage we could not justify liver biopsy. Our participants are recruited

Table 4 Univariable and multivariable analysis of the characteristics associated with fatty liver on ultrasound

Characteristics	Univariable analysis			Multivariable analysis		
	OR	95%CI for OR	*P* value	OR[1]	95%CI for OR[1]	*P* value
Age (yr)	0.99	0.98-1.01	0.55			
Male gender	0.81	0.55-1.21	0.31			
BMI (kg/m^2)	1.11	1.07-1.15	< 0.0001			
Elevated ALT[1]	4.26	2.68-6.76	< 0.0001	4.04	2.50-6.52	< 0.0001
Elevated AST[2]	2.71	1.76-4.19	< 0.0001	2.63	1.67-4.13	< 0.0001
Albumin (g/dL)	1.26	0.65-2.45	0.49	2.29	1.07-4.88	0.03
Platelets (10^9/μL)	1.00	0.99-1.01	0.14	1.00	0.99-1.00	0.55
DM[3]	2.65	1.63-4.29	0.0001	2.22	1.31-3.77	0.003
Hypertension	1.37	0.93-2.01	0.11	1.18	0.72-1.82	0.45
Hyperglycemia	3.28	2.12-5.08	< 0.0001	2.87	1.78-4.61	< 0.0001
Hypertriglyceridemia	1.92	1.30-2.83	0.001	1.82	1.21-2.75	0.004
Low HDL-C	2.43	1.64-3.59	< 0.0001	1.93	1.28-2.93	0.002
Central obesity	4.47	2.80-7.13	< 0.0001	3.52	1.94-6.39	< 0.0001
Metabolic syndrome	3.73	2.52-5.53	< 0.0001	3.33	2.07-5.01	< 0.0001
NAFLD fibrosis score						
< -1.455	1.00			1.00		
-1.455-0.676	2.05	1.37-3.06	0.0005	1.83	1.10-3.04	0.02
> 0.676	1.14	0.53-2.46	0.74	0.58	0.22-1.56	0.28
FIB4 index						
⩽ 1.3	1.00			1.00[4]		
1.31-2.66	1.19	0.76-1.85	0.45	1.40[4]	0.83-2.39	0.21
⩾ 2.67	0.59	0.19-1.85	0.37	0.69[4]	0.19-2.47	0.57
BARD						
0-1	1.00			1.00[4]		
2- 4	1.03	0.70-1.52	0.87	1.00[4]	0.65-1.52	0.99
APRI						
⩽ 0.5	1.00			1.00[4]		
0.51-1.5	2.52	1.47-4.32	0.0008	2.62[4]	1.49-4.60	0.0008
> 1.5	-	-	-	-	-	-

[1]ALT > 40 U/L for men and > 31 U/L for women; [2]AST > 37 U/L for men and > 31 U/L for women; [3]Either history of diabetes mellitus, use medication for diabetes mellitus, fasting plasma glucose ⩾ 126 mg/dL, or glycosylated hemoglobin A1C ⩾ 6.5 g/dL; [4]Adjusted for age, gender, and BMI. BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; DM: Diabetes mellitus; HDL-C: High-density lipoprotein cholesterol; APRI: AST to platelet ratio index; NAFLD: Nonalcoholic fatty liver disease.

from households and are not aware of their disease. However, given the striking findings in this small pilot sample, we now need to validate the non-invasive markers so that population based screening for early liver disease becomes feasible in high risk populations.

The series of panels, comprised of common, easily available anthropometric, hematologic and biochemical parameters were chosen based on published studies and practice guidelines supporting utility in detecting or excluding advanced fibrosis in NASH or Hepatitis C induced liver disease[7]. The NFS score (n = 733)[15], the FIB4 index (n = 541)[9], and the BARD score (n = 827)[8] were derived from largely Caucasian cohorts who underwent liver biopsy during evaluation for NAFLD. The APRI (n = 270) was derived from a cohort of patients with chronic hepatitis C who underwent biopsy[10]. Twenty to 50% of individuals in these cohorts had advanced fibrosis by biopsy and the scoring systems were useful in identifying these individuals. Applied in our cohort, the NFS, FIB4 and BARD scores predicted that between 24%-38% have indeterminate and up to 63% have high probability of NASH with advanced fibrosis. The APRI predicted 17% with indeterminate or advanced fibrosis. This high percentage of indeterminate or advanced fibrosis (73%) is surprising in a community based cohort without prior history of liver diseases. However, only the intermediate NFS and APRI scores between published low and high cut-offs correlated directly with the presence of steatosis by ultrasonography. The lack of significant association between the NFS or APRI scores higher than published high cut-off values and hepatic steatosis could be due to "burn-out" NASH as advanced liver fibrosis in NASH is often accompanied by a reduction in hepatic fat to the point of complete fat loss[17]. Alternatively, the lack of association might be attributed to type Ⅱ error due to the small number of participants who had high NFS or APRI scores. Nonetheless, the values of these scores demonstrate the need to accurately define the prevalence of advanced fibrosis in this cohort.

There are several limitations in this study. First, hepatic steatosis was detected by ultrasonography, which has decreased sensitivity with lower degrees of steatosis[5]. This approach could have resulted in misclassification of some patients with mild steatosis as having no steatosis, thereby contributing to the imprecision in diagnostic scores. However, ultrasonography is the standard technique for detecting steatosis in large clinical trials

and we prospectively performed imaging in consecutive well phenotyped participants with extensive clinical and biochemical data. The observation that over 50% of our community based cohort has steatosis despite the sensitivity limitations of ultrasound attests to the frequency of disease. In support of this we were able to utilized noninvasive panels to predict the potential burden of disease without histology. Our data are useful since these panels have been advocated for use in identifying patients at significantly increased risk for advanced fibrosis to target or avoid biopsy, respectively, and their utility in population screening has not been determined[6]. Though the potential burden of advancing liver disease looks to be high, variability and lack of concordance between panels underscore the imprecision in these measures. Our data provide a strong rationale for proceeding to liver biopsy in a sample of these individuals most at risk to directly define disease burden and to further evaluate the utility of noninvasive diagnostic strategies.

In conclusion, in this community-based study of asymptomatic Hispanics, we found a surprisingly high potential burden of NASH and advanced fibrosis. We further found that commonly used diagnostic panels employing published cut-offs, are imprecise as predictors of steatosis and NASH. We document an urgent need to identify accessible and useful screening modalities for population-based studies in Hispanics so that we can develop targeted preventive and therapeutic measures. In short, community-based prospective studies in Hispanics which include liver histology will be needed.

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COMMENTS

Background

Nonalcoholic steatohepatitis (NASH) is found in up to 20% of hispanics evaluated for hepatic abnormalities but the true prevalence in hispanic communities remains unknown.

Research frontiers

Markers that either individually or as composite panels predict the presence of NASH and advanced fibrosis of liver have been developed. These biomarkers and panels have been validated in cross-sectional studies of non-hispanic cohorts evaluated for liver abnormalities but have not been evaluated in a population setting in Hispanics.

Innovations and breakthroughs

In this population-based cohort study, the authors assessed four diagnostic panels for NASH and fibrosis in asymptomatic individuals enrolled in the Cameron County Hispanic Cohort. Based on established cut-offs for diagnostic panels, between 17%-63% of the entire cohort was predicted to have NASH with indeterminate or advanced fibrosis.

Applications

The burden of NASH and advanced fibrosis in the Hispanic community in South Texas may be more substantial than predicted from referral clinic studies. Delineation of the true prevalence of disease and validation of non-invasive diagnostic markers in this high risk population will require prospective correlation with liver histology.

Terminology

The nonalcoholic fatty liver disease (NAFLD) fibrosis score, the FIB4 index, the BARD score, and the aspartate aminotransferase to Platelet Ratio Index are non-invasive diagnostic panels that are derived from common anthropometric, hematological and biochemical parameters and are used to predict advanced fibrosis in NAFLD.

Peer-review

The study by Pan *et al* highlights the burden of NAFLD within a well defined population within the United States (*viz* the Cameron County Hispanic Cohort). The study is essentially a hypothesis generating piece of research that the researchers acknowledge requires prospective follow-up with liver histology.

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