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EDITORIAL

Role of liver resection in the management of multinodular hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of cancer related deaths worldwide. Various treatment modalities have been applied to HCC depending on the tumor load, functional capacity of the liver and the general condition of the patient. According to Barcelona Clinic Liver Cancer staging strategy and The American Association for the Study of Liver Disease guidelines, surgical resection is not advocated in the tretment of multinodular HCC. Despite this, many recent clinical studies show that, resection can achieve good results in patients with multinodular HCC and 5-year survival rate around 40% can be reached. If resection or transplantation is not performed, these patients are usually managed with palliative procedures such as transarterial chemoembolization, radioembolization and cytotoxic chemotherapy and 5-year survival of this group of patients will be extremely low. Although survival rates are lower and complications may be increased in this group of patients, liver resection can safely be performed in selected patients in experienced centers for the management of multinodular HCC.

Key words: Hepatocellular carcinoma; Hepatoma; Liver resection; Transarterial chemoembolization; Liver cancer; Liver transplantation

Core tip: Liver resection is underutilized in the management of multinodular hepatocellular carcinoma. The presence of multiple nodules should not be considered as a contraindication for surgical resection. Acceptable 5-year survival rates can be achieved in selected patients.

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TEXT

Hepatocellular carcinoma (HCC) is a major health problem and is the sixth most common malignancy in the world[1]. HCC usually develops in the presence of underlying chronic liver disease, most commonly due to chronic hepatitis B in underdeveloped countries and due to hepatitis C in developed countries. Although resection remains the cornerstone treatment for HCC, the role of resection in multinodular HCC is controversial. The early literature suggests that transplantation is the only treatment of choice that can offer long-term survival in patients with multinodular disease[2,3].

Liver transplantation eliminates not only the tumor but also the underlying liver disease and excellent outcomes with 5-year survival rates exceeding 60% can be achieved but, many patients are not candidates for transplantation because of tumor size, advanced age, high costs, and finally organ shortage[4]. Clearly, in patients with advanced underlying liver disease and portal hypertension, transplantation is the only option with a chance of cure. Tumors meeting Milan criteria defined as a single tumor smaller than 5 cm or up to three nodules smaller than 3 cm each are good candidates for liver transplantation.

Many staging systems and management algorithms have been proposed for HCC and the Barcelona Clinic Liver Cancer (BCLC) staging and treatment strategy is the most commonly used system. In the BCLC treatment algorithm, surgical resection is limited to patients with early stage single tumors. Patients with up to three nodules may be offered liver transplantation. Patients with more than three nodules are considered for palliative treatments including chemoembolization. BCLC algorithm was first introduced by Llovet *et al*[5] in 1999 in Seminars in liver disease. Since this original publication, some modifications has been emerged, but the suggestions in the management of multinodular HCC has remained unchanged[6] (Figure 1). The American Association for the Study of Liver Disease guidelines also advocates liver resection only for solitary HCC smaller than 3 cm with well-preserved liver function[7].

On the other hand, the resectability for HCC mainly depends on the volume and functional capacity of the remnant liver, but not to the resected tumor amount. The severity of underlying liver dysfunction is critical in the evaluation of patients preoperatively. During daily practice, surgeons, gastroenterogists and oncologists may see many HCC patients with good liver reserve, good general condition but with multinodular HCC. Multinodular HCC has generally been regarded as a contraindication to resection, but recently there is evolving evidence for the resection of these tumors[8-14].

Anatomic *vs* nonanatomic resection in patients with HCC is an ongoing discussion[15]. The surgeons performing resection in the treatment of multinodular HCC have to face the difficulty of achieving a curative intervention and at the same time preventing postoperative liver failure as a result of removal of too much liver. Proponents of anatomic resection claim that, eradication of intrahepatic metastasis along the portal venous system can only be achieved by systematic removal based on segmental liver anatomy[16]. On the contrary, some surgeons prefer non-anatomic resections, aiming to preserve enough tumor free margin to maximize the volume of the remnant liver. In the presence of multiple HCC nodules, anatomical resections may be difficult to perform as postoperative liver failure may be increased due to insufficient remnant liver. In an analysis of 434 patients from Japan, anatomic resections could be performed in only 36% of patients with multinodular HCC while 71% of patients had undergone anatomical resections in the presence of a single tumor[7]. Although the risk of intrahepatic disseminations is considered trivial in small tumors under 2 cm, it may be challenging to justify resection in the treatment of multinodular HCC for the anatomic resection advocates[17].

Nathan *et al*[18] analyzed the factors predictive of receipt of surgical treatment for early HCC that is, those patients with non-metastatic tumors 5 cm or smaller and without evidence of lymph node metastasis, extra-hepatic tumor growth, or major vascular invasion. Of the 1745 patients meeting the selection criteria, a total of 820 patients (47%) did not receive any type of surgical intervention. Seventy-six percent of those (n = 622) had found to have no documentation of any treatment modality in their medical records. The authors examined the factors associated with receipt of surgical therapy in a bivariate analysis. With respect to tumor characteristics, patients who receive surgical therapy generally had solitary tumors (68% *vs* 60%, $P < 0.001$) and less often had bilobar disease (14% *vs* 22%, $P < 0.001$). Surprisingly surgical treatment was not offered to many patients without any apparent reason. This Johns Hopkins University data suggest that surgical treatment options are not offered to many patients with HCC, and their opportunity of achieving better survival may be hindered.

There is not enough data about the maximum number of nodules that can be resected safely. In a recent study of 399 patients, Nojiri *et al*[19] showed that even if patients have four or more nodules without portal vein invasion and with well-preserved liver function, resection for HCC may be the treatment of choice. The 3- and 5-year overall survival rates of patients with multinodular HCC were 62% and 38% respectively. Ishizawa *et al*[8] reported 5-year survival of 58% in 126 patients with multinodular HCC and in this series 22 patients (17%) had four or more HCC nodules. In this group of patients, existence of multiple tumors was not found to be a predictor of overall survival but independently increased the risk of recurrence (relative risk 1.64)[8]. With the development of radiofrequency ablation, the combination of this modality with resection may increase the resectability rates and surgical treatment can be performed in more advanced multi-nodular HCC[20].

In a recent systematic review of 50 studies involving

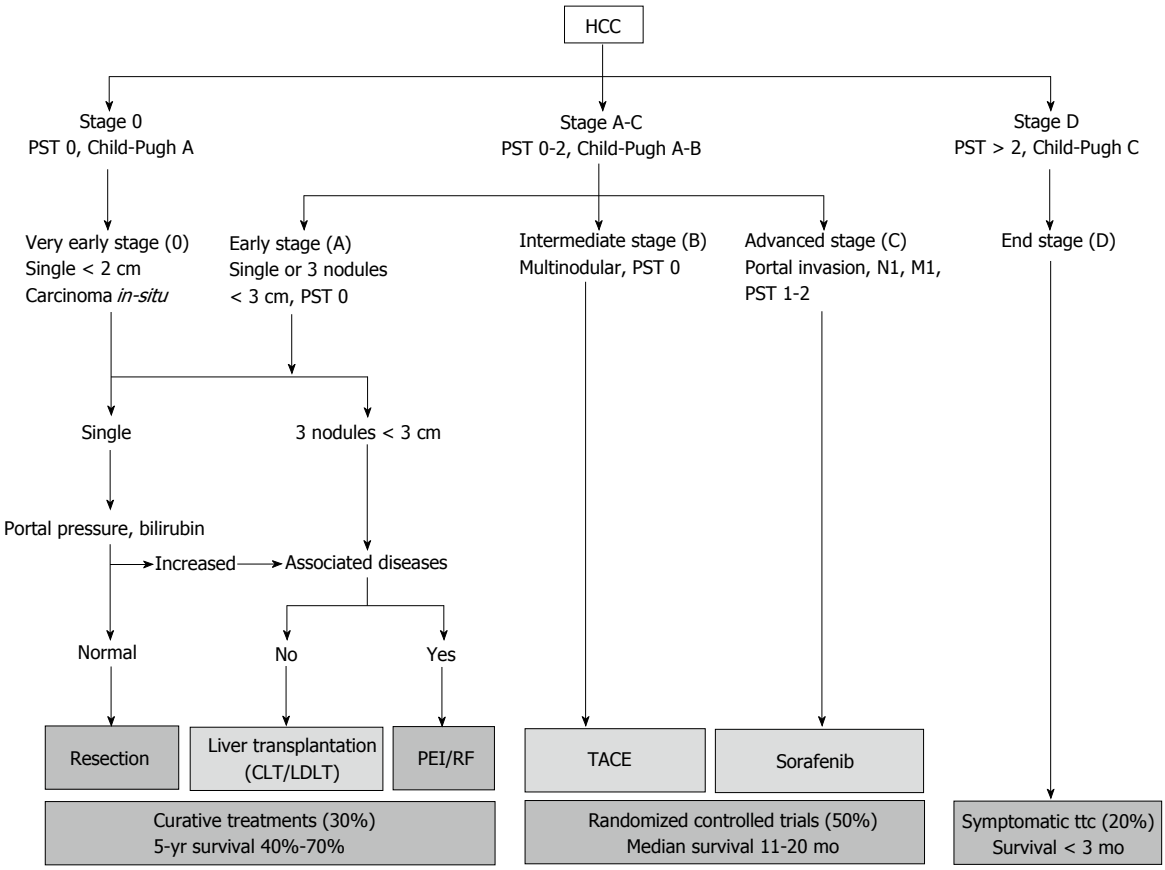

Figure 1 The Barcelona Clinic Liver Cancer staging system and treatment schedule[19]. HCC: Hepatocellular carcinoma; PST: Performance status; CLT: Cadaveric liver transplantation; LDLT: Living donor liver transplantation; PEI: Percutaneous ethanol injection; RF: Radiofrequency; TACE: Transarterial chemoembolization.

Table 1 Survival of patients with multinodular hepatocellular carcinoma in some large series

Ref.	Year	No. of patients	3-yr survival (%)	5-yr survival (%)
Nojiri *et al*[19]	2014	107	62	38
Zhao *et al*[10]	2013	266	58	ND
Ruzzenente *et al*[11]	2009	30	ND	46
Ishizawa *et al*[8]	2008	126	75	58
[1]Ikai *et al*[14]	2007	3174	48	30
Ng *et al*[12]	2005	380	50	39
Ng *et al*[12]	2005	82	43	26

[1]3 or more hepatocellular carcinoma nodules. ND: No data.

14808 patients Zhong *et al*[21] showed that resection can safely be performed in both large and multinodular HCC. In this systematic review, hospital mortality rates were found to be 2.7% to 7.3% depending on the ethnicity and type of HCC and these rates were similar to the mortality rates of early HCC surgery. The median rate of postoperative complications in this study (26.6% to 32.3%) is also comparable to early HCC series. Overall 5-year survival and 5-year disease free survival rates in these large and/or multinodular HCC patients were 42% and 26% respectively. These numbers are definitely lower than the corresponding 5-year survival of 67% and 5-year disease free survival of 37% for patients with early HCC, but still acceptable, suggesting that resection can be considered a reasonable approach in carefully selected patients.

Despite the fact that many patients with multinodular HCC may not be amenable to surgical treatment, these patients need careful evaluation for possible surgical therapy to provide that all therapeutic opportunities are being applied (Table 1).

If resection or transplantation is not offered to patients with multinodular HCC, these patients are usually managed with palliative procedures such as transarterial chemoembolization (TACE) and cytotoxic chemotherapy (sorafenib, *etc.*). Although the survival of patients after TACE is improved compared to conservative management, 5-year survival of this group of patients is still extremely low[22].

In conclusion, there is increasing evidence showing that resection can be safely extended to selected patients with multinodular HCC to achieve acceptable survival rates. Advances in the surgical treatment of HCC over the recent years have broadened the available surgical options for these patients. Although success of resection decreases in multinodular HCC, the overall 5-year survival rates approaching 40% to 50% can be achieved. With the improvements in surgical technique and perioperative management, liver resection can be considered as a safe treatment with an acceptable

mortality and morbidity rates in the treatment of multinodular HCC in experienced centers.

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EDITORIAL

Compartmentalization of hepatitis B virus: Looking beyond the liver

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Abstract

Hepatitis B virus (HBV) is classically considered to be hepatotropic, but accumulating evidences strongly support its extra-hepatotropic nature too. HBV nucleic acids and proteins have long been reported in a variety of extra-hepatic tissues. Of these, HBV has been studied in details in the peripheral blood mononuclear cells (PBMCs), due to its accessibility. From these studies, it is now well established that PBMCs are permissive to HBV infection, replication, transcription and production of infective virions. Furthermore, molecular evolutionary studies have provided definite evidences towards evolution of HBV genome in PBMCs, which is independent of evolution occurring in the liver, leading to the emergence and selection of compartment specific escape variants or drug resistant strains. These variants/resistant strains of HBV remain restricted within the PBMCs and are rarely detected in the serum/plasma. In addition, HBV infected PBMCs have been reported to be directly transmitted through intrauterine modes, and this infection does not correlate significantly with serum HBV surface antigen or HBV DNA markers. This editorial briefly reviews the current knowledge on this topic, emphasizes and delineates the gaps that are required to be filled to properly understand the biological and clinical relevance of extrahepatic tropism of HBV.

Key words: Lymphotropism; Compartmentalization; Hepatitis B virus; Peripheral blood mononuclear cell; Genotype

Core tip: This editorial discusses the phenomenon of compartmentalization of hepatitis B virus (HBV) in the peripheral blood mononuclear cells, their clinical relevance in emergence of escape mutants/drug resistant strains and also in transmission of infection through intrauterine routes. Referring to findings reported in some of the recently published articles on this topic, possible implications of compartmentalization is discussed with a focus on knowledge gaps that need to be filled to better understand HBV biology and pathology.

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TEXT

Hepatitis B virus (HBV) belongs to the family Hepadnaviridae of enveloped, partially double-stranded DNA viruses and is classically considered to be a hepatotropic virus[1]. However, HBV proteins and nucleic acids (both DNA, RNA) have been documented in a variety of extrahepatic sites, including peripheral blood mononuclear cells (PBMCs), lymph nodes, spleen, bone marrow, brain, cerebro-spinal fluid[2-7]. As compared to other tissues, extrahepatic tropism of HBV has been studied in considerable details in the PBMCs, due to their easy access. These cells have been reported to be permissive to HBV infection, replication, production of replicative intermediates and biologically competent virion particles[8-10] strongly supporting the lymphotropic nature of HBV. HBV DNA has also been found to infect bone marrow cells *in vitro*, express HBV antigens, produce virion - like particles containing HBV genome attesting to the fact that progenitor cells are also potential targets for HBV infection[11-13]. Despite the insufficiency of evidences to prove histo-pathological changes due to extrahepatic HBV infection[3,14], the significance of such tropism is enormous from the perspective of long persistence and parallel evolution of the viral genome and its transmission. Two previous case studies among liver transplant patients clearly suggested the restricted persistence of immune escape variants of HBV in PBMCs that acted as a source of re-infection[15,16].

Systematic studies on woodchuck hepatitis virus (WHV, an animal model of hepadnaviral infection), have revealed a number of unique and important facets of lymphotropism of Hepadnaviruses[17-19]. These studies have clearly demonstrated that Hepadnaviruses are strongly lymphotropic in nature and that lymphoid cells serve as an important non-hepatic reservoir for occult persistence of the virus[17,18]. Furthermore, challenge experiments with low doses of WHV was shown to induce primary occult infection, restricted within the lymphatic system, that rarely engaged the liver[18]. Such lymphoid cell restricted infection was transmissible to virus naive hosts as an asymptomatic, occult infection specifically within the lymphoid cells[18]. Interestingly, it was also demonstrated that woodchuck mothers with lymphoid cell restricted occult hepadnaviral infection transmit infection to their offspring, inducing an occult infection, that too remain restricted within the lymphatic system of the offsprings[19]. These evidences indicate a fascinating biology of lymphoid restricted Hepadnaviruses, that is distinct from hepatic infections.

Subsequently, findings resembling the WHV animal model, were found in human HBV occult infections too. A previous study from our research group reported asymptomatic, persistent occult HBV infection, specifically in the peripheral blood leukocytes (PBL), and its possible transmission within members of a family, that lacked HBV DNA in serum, clearly signifying the involvement of lymphatic cells in occult HBV infection[20]. Based on the analysis of HBV sequences isolated from the PBLs, it was observed that despite the presence of two different subtypes of HBV, namely *"ayw"* and *"adw"* (genotypes D and A, respectively) in the family, only subtype *adw* with an immune escape mutation of HBV surface antigen (HBsAg) (G145R) was present in the PBLs of all the family members, that possible acquired HBV by non-sexual intrafamilial modes[20]. The results of this study also suggested the different modes of transmission of HBV subtypes, *i.e.*, possible sexual transmission for *"ayw"* and restricted persistence of *"adw"* with G145R within the PBL and its transmission through non-sexual modes. Later, we demonstrated the PBL specific persistence of HBV subgenotype Ae/A2 with G145R even in unrelated individuals within our study population[8]. Using multiple clonal analyses of HBV DNA from serum and PBL from the same individuals, we detected diverse HBV subgenotypes (D1, D2, D3, D5, Cs/C1 and Aa/A1) in the serum, but could not detect subgenotype Ae/A2 sequences in any of the serum samples analyzed. On the other hand HBV subgenotype Ae/A2 with G145R was exclusively present in the PBL of majority of the subjects, signifying the compartmentalization of a typical HBV type with immune escape variants across a population of unrelated individuals, as previously reported for other viruses too[21-23]. It has long been recognized that HBV interacts with cell receptors present on the hepatocytes and lymphocytes through its preS envelope protein, and amino acid residues 21-47 are crucial for this interaction[24]. Interestingly, from the analysis of HBV multiple amino acid sequences, it has been observed that the length of the preS region vary among HBV genotypes, and also that the preS region is remarkably conserved within genotypes in relation to its marked inter-genotype variability[25]. These facts suggest the HBV genotype specific differences in attachment efficiency to cellular receptors present on diverse cell types, and might be responsible for genotype specific compartmentalization of HBV. Despite being discovered much later than HBV, in sharp contrast to HBV, compartmentalization have been well studied for many other DNA and RNA viruses, including human immunodeficiency virus (HIV), HCV, epstein barr virus[26-29].

In the recent years, studies on genetic variability of HBV in PBMCs and in paired liver/plasma from different groups of HBV infected individuals, have provided strong evidences in support of compartmentalized evolution of HBV within the PBMCs[30,31]. In these studies, researchers have investigated the HBV genetic variability, drug resistance and immune escape mutation patterns in plasma and PBMCs from patients in different phases of the chronic hepatitis B (CHB). Interestingly, in one

study on 22 patients, only 3 patients had identical HBV genotype profiles in plasma and PBMCs[7]. Moreover, the occurrence of immune escape mutations was also found to be mostly compartment specific, being frequently detected in the PBMCs of immune-active CHB patients[7]. Similarly, in another recent study on HIV-HBV co-infected individuals, researchers documented compartment-specific evolution of HBV, as evident by distinct resistance mutation profiles in the plasma and cerebro-spinal fluid, signifying independent evolution of HBV in the central nervous system[32]. Infection of immunologically privileged sites by different viruses, evolution of escape variants is known to be a well recognized immune evasion or immune modulation strategy, well recognized in case of other viruses such as HCV[33] and HIV[34-37].

Apart from providing a privileged site for viruses to persist and evolve, PBMCs also play an important role in virus transmission, through trafficking of maternal PBMCs to the fetal blood[38]. More specifically, recent studies have demonstrated *in utero* transmission of HBV (including vaccine escape mutants) *via* PBMCs, crossing the placental barrier[39,40]. In a recent study on PBMC HBV DNA positive subjects, the authors observed that HBV infected PBMCs from the mothers are able to cross the placental membrane, and infect the fetus[40]. Very recently, a similar study, reported mother-to-infant PBMC trafficking activity in 63% of the study subjects and intrauterine transmission of HBV through this trafficking of infected PBMCs was evident in 71.4% of the neonates[41]. The intrauterine infection rate was much higher in neonates born to PBMC HBV DNA-positive mothers, as compared to PBMC HBV DNA-negative mothers. The results of this study clearly demonstrated that mother to fetal PBMC traffic significantly increased the risk of PBMC HBV infection in newborns. However, surprisingly, no noteworthy association was found between mother to fetal HBV positive PBMC transfer and detection of serum HBsAg and/or HBV DNA positivity in the newborns[41] signifying that mother to fetus transfer of HBV positive PBMCs is not frequently reflected in serum. Additionally, the response to therapeutic approaches has been shown to be different in PBMC restricted HBV, as compared to HBV persisting in the liver[30,31]. Thus, there remains a serious concerns regarding the use of therapeutic approaches for prevention of vertical transmission of HBV, since serum marker based evaluation studies might not represent the actual incidences of transmission of HBV infected PBMCs or the efficacy of therapeutic interventions in containing such transmissions.

From the accumulating data, it is gradually becoming apparent that infection of lymphocytes is an inevitable phenomenon in a number of viral infection, including HBV. Interestingly, this also raise a serious question, if HBV is really a classical hepatotropic virus. Perhaps, further studies in this direction might lead to the answer in future. Nevertheless, persistence of HBV in PBMCs have important implications in long term persistence, emergence of immune escape/drug resistant variants and also in transmission. It is thus extremely essential to study the phenomenon in details to properly understand the mechanisms involved. Further deliberations might be necessary to recommend testing of PBMCs for routine diagnosis of HBV infection, particularly in studies related to monitoring of transmission or therapeutic efficacy. Taking into account the significance of PBMCs in transfusion, transplantation, therapeutics, vaccination, and intrauterine transmission, a comprehensive understanding of HBV infection in these cells is imperative for designing effective strategies to reduce the burden of HBV and other viral infections.

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REVIEW

Evaluation of antiangiogenic efficacy in advanced hepatocellular carcinoma: Biomarkers and functional imaging

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Abstract

Many years after therapeutic wilderness, sorafenib finally showed a clinical benefit in patients with advanced hepatocellular carcinoma. After the primary general enthusiasm worldwide, some disappointments emerged particularly since no new treatment could exceed or at least match sorafenib in this setting. Without these new drugs, research focused on optimizing care of patients treated with sorafenib. One challenging research approach deals with identifying prognostic and predictive biomarkers of sorafenib in this population. The task still seems difficult; however appropriate investigations could resolve this dilemma, as observed for some malignancies where other drugs were used.

Key words: Hepatocellular carcinoma; Antiangiogenic therapies; Sorafenib; Predictive biomarkers; Prognosis biomarkers; Functional imaging

Core tip: The approval of sorafenib in advanced hepatocellular carcinoma is based on the positive results of two large randomized phase Ⅲ clinical trials. The inter- and intra-individual variability regarding tumor response and clinical outcome highlighted the unmet need of effective biomarkers of response. These biomarkers could be useful for monitoring treatment activity, detecting early resistance to treatment and identifying patients who would more likely benefit from treatment. An overview of prognostic/predictive biomarkers of sorafenib in hepatocellular carcinoma is discussed in this review.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide[1,2]. The incidence of HCC is steadily increasing with about 625000 new cases per year and the disease results in around 600000 deaths yearly over the world[1,2]. Less than 30% of patients diagnosed with HCC are eligible for curative treatment[3] and during the course of the natural evolution of HCC; a significant proportion of patients are candidates for systemic therapies. In recent years, considerable progress has been made in furthering the knowledge of molecular biology of HCC, including better understanding of the role of signaling pathways and angiogenesis[4-8]. These advances have led to the development of targeted therapies in HCC[9-11]. Nevertheless, only sorafenib, a multikinase inhibitor, remains till date the sole approved drug in advanced HCC, based on the clinical benefit observed in properly selected patients enrolled in clinical trials[12,13]. With only three months of survival gain compared to placebo, many practitioners and country health authorities consider the cost-efficacy ratio of sorafenib somewhat insufficient[14-16]. In some emerging countries, the drug is not even approved for patients with advanced HCC. Otherwise, published data and clinical practice highlight a great inter-individual and even intra-individual variation regarding clinical benefit and toxicity[17-22]. For clinicians, there is an unmet need to identify patients more likely to benefit from treatment. Thus, to dispose of predictive markers of response and to support the decision to continue treatment when better outcome has been detected early. Thus, to improve patient management, avoid side effects when sorafenib has proved ineffective, and control health expenses and clinical research. Numerous clinical, plasma and tumor-derived biomarkers have already been studied. Some of them have been proposed as predictive surrogate markers of activity of sorafenib and other antiangiogenic agents. Furthermore, Response Evaluation Criteria in Solid Tumors (RECIST) criteria[23,24] were proposed to evaluate tumor size changes during treatment in patients with cancer. Novel imaging techniques and radiological methods were suggested to strengthen the standard RECIST criteria in HCC to evaluate, directly in patients, the effects of drugs on tumor angiogenesis.

Herein, we review the current knowledge about prognostic/predictive and pharmacodynamics biomarkers for sorafenib and other antiangiogenic agents in advanced HCC and their potential integration into clinical practice. We also discuss the place of functional imaging to evaluate tumor response in advanced HCC. The Tables 1-3 give an overview of different studies of biomarkers in advanced HCC referred to in this review.

BIOMARKERS

Definitions, why biomarkers?

The national institute of health defined "biological marker (biomarker): a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention"[25]. Additionally, Ludwig *et al*[26] defined biomarkers as molecular, cellular or functional quantifiable or quantitative parameters indicative of particular genetic, epigenetic histological or cytological tumor abnormality. Initially, biomarkers were used for risk assessment and screening in cancers and later, to enhance cancer staging, to refine prognosis and to evaluate the response to biological therapy[27]. Biomarkers could then be clinical, biological, molecular or imaging parameters. Identifying prognostic and predictive biomarkers to antiangiogenic therapies is a crucial issue in HCC to be integrated into clinical care in the future. Previously, some predictive biomarkers of anticancer therapy response were identified in the field of oncology. Indeed, the efficacy of anti-epidermal growth factor receptors, such as cetuximab and panitumumab, in metastatic colorectal cancer is limited to proto-oncogene proteins p21(ras) (KRAS) wild-type cancer[28-30]. Other predictive biomarkers are used in clinical practice. For instance, the human epidermal growth factor receptor 2 expression in gastric and breast cancers to predict response to trastuzumab[31-33] and pertuzumab[34]. Moreover, gefitinib and erlotinib showed significant efficacy in patients with specific endothelial growth factor receptor (EGFR) mutations[35,36]. Recently, proto-oncogene proteins B-raf (BRAF) V600 E mutation in patients with metastatic melanoma was proved to be predictive of response to vemurafenib[37]. Regarding HCC, biomarkers should ideally meet at least the following criteria[26,38]: (1) to be easily measurable through minimally invasive procedures, ideally using blood tests; (2) to have a prognostic value in relation to the natural history and the outcome of HCC; (3) to have a predictive value wherein its presence correlates with the clinical response to sorafenib therapy; and (4) preferably not to be detectable in premalignant diseases (*e.g.*, cirrhosis).

Clinical biomarkers

Positive impact of drug-related cutaneous adverse events on clinical outcome was initially reported in patients treated with epidermal growth factor receptor inhibitors for advanced colorectal cancers[29,39], non-small-cell lung cancers[40] and pancreatic cancers[41]. Some retrospective studies have shown in patients with advanced HCC treated with sorafenib a positive association with early skin drug-related toxicities and clinical benefit[42-44] and

Table 1 Association between baseline circulating markers and outcome in patients treated with various treatments for hepatocellular carcinoma

Ref.	Markers	Patients (*n*)	Study design	Treatment	Level values	Clinical impact	Conclusion/comments
Schoenleber *et al*[85]	VEGF-A	1018	Systemic review and meta-analysis including only serum-based studies	Various (surgery, LRT and systemic therapies)	High serum VEGF level	Poorer OS Poorer DFS	Serum VEGF method detection varied among studies Serum VEGF levels seem more reliable than tissue VEGF for HCC prognosis
Poon *et al*[115]	bFGF	88	Prospective	Surgery	High serum level > 10.8 pg/mL	Larger tumor > 5 cm Venous invasion	High bFGF serum level before surgery was shown to be an independent factor of early recurrence. No further studies confirmed these findings
Vejchapipat *et al*[105]	HGF	55	Retrospective	BSC	High level (≥ 1.0 ng/mL	Advanced pTNM stage Poorer prognosis Poorer OS	Although a control group was included, results of this small cohort study need confirmation in larger prospective analysis
Chau *et al*[104]		40	Retrospective	Resection	High portal and serum HGF level (> 699 pg/mL)	Multiple tumor Poorer prognosis	One limit of this study were the feasibility in routine of intraoperative puncture of the portal vein was difficult
Mizuguchi *et al*[106]		100	Retrospective	Resection	High serum level (≥ 0.35 ng/mL)	Postoperative complications Poorer OS	No correlation was observed between HGF level and RFS
Kaseb *et al*[87]	IGF-1	288	Prospective	Various	Low plasma level (26 ng/mL)	High Child-Pugh score High AST level High tumor size Multiple tumor Vascular invasion Poorer OS	The authors proposed that IGF-1 plasma level to be integrated into the BCLC staging system to predict OS for personal management in patients with HCC. This proposal was not yet adopted in clinical practice

BCLC: Barcelona clinic liver cancer; bFGF: Basic fibroblast growth factor; BSC: Best supportive care; DFS: Disease-free survival; HGF: Hepatocyte growth factor; IGF-1: Insulin growth factors 1; LRT: Loco-regional treatment; OS: Overall survival; RFS: Recurrence-free survival; VEGF: Vascular endothelial growth factors.

disease control[44,45] (Table 4). Recently, the Barcelonan group reported the results of a prospective single-arm, monocentric study that assessed the link between early sorafenib-related skin toxicities and outcome in patients with advanced HCC[46]. Added to baseline performance status and barcelona-clinic-liver-cancer staging system[47], early sorafenib-induced skin reactions were an independent predictor of overall survival (OS). Patients who experienced skin adverse events have a better outcome compared to patients without any cutaneous reactions. The time to progression (TTP) was significantly longer in the first group (8.1 mo, 95%CI: 1.6-14.5, *vs* 3.9 mo, 95%CI: 2.08-5.7; $P = 0.016$) as well as OS (18.2 mo, 95%CI: 11.9-24.4, *vs* 10.1 mo, 95%CI: 10.1-13.0; $P = 0.009$)[46]. Accordingly, early skin reactions during sorafenib treatment may indicate antitumor effect and clinical benefit in patients with advanced HCC. These findings support the need to maintain treatment provided that these side effects are well managed.

Arterial hypertension is a frequent side effect observed in patients treated with antiangiogenic agents. The incidence of arterial hypertension in patients treated with sorafenib for advanced cancers was estimated at 23.1%[48]. Previous studies showed a positive link between arterial hypertension due to bevacizumab and outcome in patients with advanced colorectal cancer[49,50] and renal cell cancer[51] or related to axitinib in pancreatic cancer[52]. However, a recent systematic review of all placebo-controlled phase Ⅲ trials with bevacizumab failed to demonstrate any positive impact of drug-related arterial hypertension and clinical benefit [progression-free survival (PFS) and OS] in patients with advanced cancers[53]. Sorafenib-induced arterial hypertension was reported to be predictive of clinical benefit in patients with metastatic renal cell cancer[54]. Estfan *et al*[55] found in a small cohort of patients with advanced HCC that arterial hypertension related to sorafenib correlated with better OS[55]. These results were not reproduced in other retrospective[42] and prospective[46] studies. Thus, no robust data is available to prove the link between an increase in blood pressure during sorafenib treatment and clinical benefit or antitumor activity for HCC (Table 4). In summary, no clinical biomarkers of response to sorafenib were validated in clinical practice. Based on the Barcelonan prospective study, cutaneous adverse events seem to be the best track to explore in patients treated with sorafenib for advanced HCC. These results should be interpreted with caution since no untreated control arm was evaluated in this study.

Circulating biomarkers

Alpha-fetoprotein: Serum alpha-fetoprotein (AFP) is the only biomarker that passed all five phases of

Table 2 Prognostic value of baseline circulating factors in patients treated with systemic therapies including antiangiogenic agents for advanced hepatocellular carcinoma

Ref.	Marker	Patient (*n*)	Study type	Treatment	Levels values	Prognostic value	Conclusion/comments
Kaseb *et al*[86]	VEGF-A	394	Systemic review including only serum or plasma-based studies	Various (AA alone or combined with CT)	High serum or plasma level	Poorer outcome	Plasma VEGF seemed more relevant than serum VEGF as prognostic factor for HCC
Llovet *et al*[63]		490	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	High plasma level (> 101 pg/mL)	Poor OS Better clinical/ demographic parameters	The VEGF level was a prognostic factor for all patient's cohort but surprisingly it did not affect prognosis in patients receiving sorafenib. Moreover, the VEGF level did not predict response
Llovet *et al*[63]	HGF	251	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	High plasma level	Poorer OS	HGF was a prognostic factor for the entire cohort. However, it does not predict response to sorafenib (only a nonsignificant trend)
Miyahara *et al*[112]	Ang2	30	Prospective?	Sorafenib	High serum level	Shorter PFS Progressive disease	The small cohort and the lack of control arm hamper conclusion on the role of Ang2 as predictive of response to sorafenib
Llovet *et al*[63]		490	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	High plasma level (> 6043.5 pg/mL)	Poorer OS Better clinical/ demographic parameters	Ang2 was shown to be a prognostic factor in HCC but did not predict response to sorafenib
Llovet *et al*[63]	c-KIT	245	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	High plasma level (> 11.3 ng/mL)	Trend to a better OS Trend to better TTP Better clinical/ demographic parameters	Soluble c-KIT was shown to be a prognostic factor for HCC. However, it showed only a nonsignificant trend to predict response to sorafenib
Llovet *et al*[63]	IGF-2	254	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	High plasma level (> 797.7 ng/mL)	Better OS Better clinical/ demographic parameters	IGF-2 was shown to be prognostic factor in HCC but did not predict response to sorafenib
Shao *et al*[126]	CEC/ CECP	40	Prospective	Sorafenib + CT	High CECP level	Poorer PFS Poorer OS	The predictive value of CECP was not confirmed in further investigations

AA: Antiangiogenic; Ang2: Angiopoietin 2; CEC: Circulating endothelial cells; CECP: Circulating endothelial cell progenitors; c-KIT: Stem-cell factor receptor; CT: Chemotherapy; HCC: Hepatocellular carcinoma; HGF: Hepatocyte growth factor; IGF-2: Insulin growth factor 2; OS: Overall survival; PFS: Progression-free survival; TTP: Time to progression; VEGF: Vascular endothelial growth factors.

biomarker development as defined by Pepe *et al*[56]. AFP remains a useful prognostic marker and probably a predictive marker of treatment response in HCC (Tables 5 and 6). In a large Chinese retrospective cohort, high serum AFP level correlated with larger HCC size, vascular invasion and low tumor differentiation[57]. Previous studies showed that AFP levels could be useful to predict recurrence after surgery[58,59], liver transplantation[60-62]. The value of AFP as a prognostic marker was reported in several studies evaluating sorafenib in advanced HCC. The SHARP trial[12] is a phase 3, placebo-controlled trial that studied the benefit of sorafenib *vs* placebo in 602 patients with advanced HCC. Llovet *et al*[63] showed in patients included in this study that high baseline AFP plasma levels (> 200 ng/mL) have a negative impact on OS[63]. These findings confirmed previous results reported with sorafenib a small cohort of patients with advanced HCC[64], in retrospective analysis[65]. High baseline serum AFP level (≥ 400 ng/mL) also seemed associated with shorter TTP[63]. Noticeably, in a recent analysis of six prospective phase Ⅱ trials evaluating systemic therapies for patients with advanced HCC, no association between baseline AFP levels and prognosis was observed[66]. More interestingly, some authors evaluated the kinetics of AFP during treatment in HCC as a predictive marker of response or outcome. Previous studies showed a positive correlation between the decrease of AFP plasma levels and objective response and OS in patients with advanced HCC receiving systemic therapies[67,68]. Small series reported the value of baseline and changes in AFP plasma levels to predict response and outcome for patients with advanced HCC treated with sorafenib. Several studies showed consistent correlation between early (varying from 2 to 8 wk) decrease of AFP level more than 20% following sorafenib and objective response[69-73] and better outcome[69-71,73] in patients with advanced HCC. Personeni *et al*[71] showed that early responders, defined by a 20% decrease of AFP 8 wk after sorafenib treatment, had significantly better median OS and TTP compared to non-responders (13.8 mo *vs* 8.2 mo, P = 0.022 and 7.9 mo *vs* 2.4 mo, P = 0.004; respectively)[71]. In a recent study, Nakazawa *et*

Table 3 Treatment-induced changes in biomarkers levels and association with outcome in patients with hepatocellular carcinoma

Ref.	Marker	Patient (*n*)	Study design	Treatment	Marker treatment-induced changes	Impact value	Comments
Llovet *et al*[63]	VEGF-A	490	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	Increase	No association with OS and ORR	The VEGF-A could serve as pharmacodynamic marker of exposure to sorafenib but did not have prognostic or predictive value
Harmon *et al*[93]		37	Prospective single arm phase Ⅱ	Sunitinib	Reversible Increase	Better DCR Better PFS Better OS	Inconsistent results were observed in these trials.
Zhu *et al*[91]	VEGF-C	34	Prospective single arm phase Ⅱ	Sunitinib	Sustained increase	No predictive value	The value of VEGF-A to predict response to sunitinib could be confirmed in larger trial
Harmon *et al*[93]		37	Prospective single arm phase Ⅱ	Sunitinib	Decrease	Better DC Better ORR	The predictive value of VEGF-C was not shown for sorafenib probably because of its limited action against the VEGFR-3
Harmon *et al*[93]	sVEGFR-2/ sVEGFR-3	37	Prospective single arm phase Ⅱ	Sunitinib	Reversible decrease	Better OS (for sVEGFR-2)	The small cohort did not allow a definite conclusion
Zhu *et al*[91]		34	Prospective single arm phase Ⅱ	Sunitinib	Decrease	No predictive value	
Llovet *et al*[63]	Ang2	490	Prospective phase Ⅲ trial	Sorafenib *vs* placebo	No significant change (for sorafenib) Increase (for placebo)	Shorter TTP Shorter OS (for patients who experienced increase)	Ang2 was probably a prognostic biomarker than predictive of response to sorafenib
Llovet *et al*[63]	c-KIT	245	Prospective single arm phase Ⅱ	Sorafenib *vs* placebo	Decrease (sorafenib) no change (placebo)	No predictive value	Tumor expression of KIT was considered as low in HCC, and the role of soluble KIT remains unclear
Zhu *et al*[91]		34	Prospective single arm phase Ⅱ	Sunitinib	Decrease	Better TTP Better OS	
Harmon *et al*[93]		37	Prospective single arm phase Ⅱ	Sunitinib	Decease	Better TTP	
Boige *et al*[98]	CEC	36	Prospective single arm phase Ⅱ	Bevacizumab	Early increase	Better OR Better DCR	CEC level was not associated with prognosis in this study. However, it could predict response to bevacizumab. The rarity of CEC level and non-standardized measurement methods limited the use of CEC as a predictive marker of response to treatment in HCC
Zhu *et al*[91]	CECP	34	Prospective single arm phase Ⅱ	Sunitinib	Decrease	Progression	

Ang2: Angiopoietin 2; CEC: Circulating endothelial cells; CECP: Circulating endothelial cell progenitors; c-KIT: Stem-cell factor receptor; DCR: Disease control; HCC: Hepatocellular carcinoma; ORR: Objective response; OS: Overall survival; PFS: Progression-free survival; sVEGFR: Soluble vascular endothelial growth factors receptor; TTP: Time to progression; VEGF: Vascular endothelial growth factors.

al[74] did not find a significant link between pretreatment AFP levels and tumor response in patients with advanced HCC treated with sorafenib. However, an early increase in AFP levels correlates with poorer outcome with shorter OS and PFS[74].

Japanese groups proposed the lens culinaris agglutinin reactive AFP (AFP-L3), an isoform of AFP, as a good diagnostic and prognostic biomarker for HCC[75-77]. However, scant data is/are available regarding the value of AFP-L3 as predictive of response to antiangiogenic agents in HCC[78].

In summary, available data are not consistent enough to confirm the value of baseline AFP level as a predictive marker of response to antiangiogenic treatment for patients treated for advanced HCC[79].

Des-gamma-carboxy prothrombin: Des-gamma-carboxy prothrombin (DCP) is a prognostic factor for HCC as shown by Japanese research[80]. Changes in DCP plasma level were evaluated in patients treated with sorafenib[73,81,82]. Some studies reported that DCP could be an independent factor of survival in patients

Table 4 Clinical side effects induced by sorafenib in patients with advanced hepatocellular carcinoma and association with outcome

Ref.	Side effect	Patients (*n*)	Study design	Impact on survival	Impact on other parameters	Predictive value
Otsuka *et al*[42]	Skin reaction	94	Retrospective	Better OS	No impact on ORR, DCR, and TTP	No
Vincenzi *et al*[45]		65	Retrospective	Trend to a better OS	Better DCR Better TTP	Early skin toxicity could predict efficacy of sorafenib
Di Costanzo *et al*[43]		65	Retrospective	Better OS	Not reported	Skin toxicity could predict survival
Shomura *et al*[44]		37	Retrospective	Better OS	Better DCR	Skin toxicity could predict efficacy
Reig *et al*[46]		147	Prospective	Better OS	Better TTP	Early skin reaction could predict efficacy of sorafenib and survival
Otsuka *et al*[42]	Arterial hypertension	94	Retrospective	No impact	No impact	No
Estfan *et al*[55]		41	Retrospective	Better OS	Trend to better TTP	

DCR: Disease control rate; OS: Overall survival; ORR: Objective response rate; TTP: Time to progression.

Table 5 Prognostic value of baseline and increase of alpha-fetoprotein for hepatocellular carcinoma in patients who underwent resection or transplantation

Ref.		Patient (*n*)	Study design	Treatment	Level values	Impact value	Comments
Liu *et al*[57]	AFP	2034	Retrospective	Resection (79.2%) NA (20.8)	High AFP levels (> 20 μg/L)	Large tumors (≥ 10 cm) Higher vascular invasion Lower differentiated tumor	This large cohort study showed that High AFP level was associated with poor prognosis and poor clinicopathological features of HCC
Wang *et al*[139]		160	Retrospective	Resection	High AFP level (> 4000 UI/L)	Shorter median TTR	In this study, the value of AFP levels to predict recurrence is limited since only a few numbers of patients (9%) have AFP level higher than the cutoff level
Ma *et al*[58]		108	Retrospective	Resection	High AFP level (> 20 ng/mL)	Lower differentiated tumor Higher vascular invasion Higher postoperative 2-yr recurrence rate Lower 24-mo survival rate	This study demonstrated the negative impact of high AFP levels on surgery benefit and the need to closely screen patients after resection for recurrence
Ikai *et al*[59]		12118	Japanese nationwide Analysis Comparative study	Resection	High AFP level (≥ 20 ng/mL)	Worsen OS after surgery	This large cohort study showed better outcome of patient resected for HCC in the last decade but the persistence of the negative impact of high AFP level on prognosis
Vibert *et al*[60]		153	Retrospective	LT	AFP level increase > 15 μg/L per month	Lower OS Lower RFS Higher recurrence rate	This study showed the negative impact on the outcome of AFP levels increases in patients undergoing LT
Hakeem *et al*[61]		12159	Systemic review	LT	AFP > 1000 ng/mL (based on the majority of study included in the review)	Poorer OS Poorer DFS Higher vascular invasion Poorer differentiated tumor	The authors stressed the poor quality of previous studies and the need for high-quality evidence on outcomes to use AFP levels as a prognostic indicator for patients undergoing LT
Duvoux *et al*[62]		972	Prospective/ retrospective	LT	High AFP level	Tumor recurrence Vascular invasion Poor differentiation	A new score model including AFP level was proposed to select patients for LT

AFP: Alpha-fetoprotein; DFS: Disease-free survival; HCC: Hepatocellular carcinoma; LT: Liver transplantation; NA: Not available; RFS: Recurrence-free survival; TTR: Time to recurrence.

treated with sorafenib[81,82]. These results were not reproduced in other reports[73]. DCP is currently used mainly in Japan and should be investigated more in a western HCC population.

Vascular endothelial growth factors: The vascular endothelial growth factors (VEGF) is one of the potent pro-angiogenic factors implicated in cancer angiogenesis. The activation of the complex VEGF/VEGF receptor (VEGFR) stimulates endothelial cell growth, proliferation, invasion and survival[83]. Circulating VEGF level may be useful in evaluating VEGF expression in HCC tumor[84] and were found suitable for HCC prognosis[85]. The VEGF-A isoform promotes angiogenesis and the dual VEGF-C/VEGF-D isoforms stimulates the lymphangiogenesis through activation of

Table 6 Prognostic and predictive value of baseline or changes of alpha-fetoprotein level for patients with hepatocellular carcinoma treated with antiangiogenic therapies alone or combined with systemic therapies

Ref.		Patients (*n*)	Study design	Treatment	Level values	Clinical impact	Comments
Shim *et al*[160]	AFP	57	Retrospective	Sorafenib	High level ⩾ 400 ng/mL	Shorter TTP	This study suffers from some limits: a retrospective study, a small cohort including only hepatitis B patients, short median follow-up duration, lack of correlation with OS or ORR
Shao *et al*[69]		72	Prospective	Various AA + CT	AFP response (> 20% decrease from baseline within the first four weeks)	Better DCR Better ORR Better PFS Better OS	The magnitude of AFP decline (20% or 50%) from baseline was not clearly defined. Similarly, the time point for evaluation of AFP level was not clear also (4 wk? 7 wk?). Limits: a small number of patients with heterogeneous treatment
Yau *et al*[70]		94	Retrospective	Sorafenib	AFP response (> 20% decrease from baseline within the first six weeks)	Clinical benefit rate Better PFS Marginal better OS	The cutoff value to define AFP response was inconsistent between various studies
Personeni *et al*[71]		85	Retrospective	Sorafenib	AFP response (> 20% decrease from baseline within the first six weeks)	Better DCR Better TTP Better OS	The authors used the landmark method to limit the potential favorable outcome due to tumor features than to AFP response
Køstner *et al*[72]		76	Retrospective	Sorafenib	AFP response (> 20% decrease from baseline within the first four weeks)	Better ORR	No correlation was observed between AFP response and OS probably because of the limited number of patients evaluated and the unusual poor OS seen in all cohort (5.4 mo)
Kuzuya *et al*[73]		48	Retrospective	Sorafenib	AFP response (decrease from baseline within 2 and 4 wk)	Better DCR Better TTP Better OS	Limits of the study: retrospective design and the small number of patients included
Nakazawa *et al*[74]		59	Retrospective	Sorafenib	AFP response (increase from baseline within four weeks)	Progressive disease Shorter PFS Shorter OS	Limits of the study: a small number of patients was enrolled in this and retrospective study. No association between AFP level before treatment and tumor response was observed
Llovet *et al*[63]		491	Prospective Phase Ⅲ trial	Sorafenib vs placebo	High plasma level > 200 ng/mL	Poorer OS	The impact of baseline AFP on survival was observed in both groups of patients treated with placebo or sorafenib
Hsu *et al*[64]		53	Prospective single-arm Phase Ⅱ trial	Sorafenib + mT/U	> 400 ng/mL	Poorer OS?	The prognostic value of baseline AFP level was shown only in univariate analysis and only score CLIP ⩾ 3 was an independent prognostic factor of poor OS
Baek *et al*[65]		201	Retrospective	Sorafenib	⩾ 400 ng/mL	Shorter FFS Poorer OS	Baseline AFP level, tumor size, PS, albumin and bilirubin levels were the independent factor associated with OS in this study
Lin *et al*[66]		156	Systemic review of the prospective phase Ⅱ trials	Various systemic therapies	⩾ 400 ng/mL	No impact	Limits of the study: heterogeneous population
Shao *et al*[119]		45	Pooled analysis of single-arm phase Ⅱ trials	Sorafenib + mT/U and beva + C	> 400 ng/mL	No impact	This study especially focused on the impact of IGF factors on outcome and the small cohort analyzed limits the interpretation of the effect of AFP levels on survival

AA: Antiangiogenic; AFP: Alpha-fetoprotein; Beva: Bevacizumab; C: Capecitabine; CLIP: Cancer of the liver Italian program[161]; CT: Chemotherapy; DCR: Disease control rate; FFS: Failure-free survival; mT/U: Metronomic tegafur/uracil; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; PS: Performance status; TTP: Time to progression.

the VEGFR-2 and VGEFR-3 respectively. Several studies showed that high baseline levels of VEGF-A impacts negatively on prognosis in patients with advanced HCC[63,85-87]. Ebos *et al*[88] demonstrated that monitoring of soluble VEGFR-2 (sVEGFR-2) in mouse tumor models could be suggestive of the overall circulating VEGF levels and therefore, a potential surrogate biomarker for VEGF-dependent tumor growth[88]. An inverse link between sVEGFR-2 plasma levels and tumor size was detected. Recently, sVEGFR-1 levels were shown to be associated with more advanced-stage HCC and tumor differentiation and sVEGFR-2 levels to be associated with

poorly differentiated tumor[89]. Llovet *et al*[63] reported changes of plasma VEGF level in patients treated for HCC enrolled in the SHARP study. Compared to baseline level, a significant increase in plasma level of VEGF was observed in the sorafenib group (P = 0.010) and a significant decrease in plasma level of sVEGFR-2 and sVEGFR-3 was seen in the placebo group (P < 0.0001)[63]. The increase of VEGF plasma level found after sorafenib treatment was somewhat surprising since sorafenib showed OS improvement. However, similar findings were observed in patients treated with sorafenib for renal cell carcinoma[90], with sunitinib for advanced HCC[91-93] or renal cell carcinoma[94-96]. Increase of VEGF plasma level could be subsequent to hypoxia induced by the antiangiogenic agents[94]. Noticeably, a reversible increase in the VEGF level induced with sunitinib was also observed in non-tumor-bearing mice suggesting a systemic response that possibly masks tumor-specific changes or any difference in responding patients. Therefore, the increase in VEGF in response to treatment could also occur independently of tumor[97] and might explain the absence of correlation between this change and the outcome in HCC patients treated with antiangiogenic agents[63]. In the SHARP trial, the increase of VEGF-A plasma concentration during sorafenib treatment observed in patients with advanced HCC did not predict OS or tumor response[63]. Similarly, no association between VEGF-A plasma level changes and outcome was observed in patients treated with bevacizumab for advanced HCC[98]. Accordingly, the VEGF-A could serve as a pharmacodynamic marker of exposure to antiangiogenic agents but did not have prognostic or predictive value[85]. Sunitinib induced in patients with HCC, a reduction of VEGF-C (the ligand of VEGFR-3) plasma level that was associated with disease control and tumor response according to the RECIST criteria[23] and Choi criteria[99,100] respectively[93]. Likewise, sunitinib-induced decrease of sVEGFR-3 plasma levels in patients with renal cell cancer and breast cancer correlated with a better outcome[95,101]. Baseline level of VEGF-C may be regarded as a potential predictive biomarker of sunitinib efficacy in patients with advanced HCC[92,93]. However, as sorafenib has limited action against the VEGFR-3[102], the value of this biomarker to predict response in HCC patients could be anecdotal.

In summary, further robust studies are warranted to demonstrate the predictive value of circulating VEGF in patients treated with sorafenib or other antiangiogenic agents for advanced HCC. The plasma VEGF should be assessed more than serum VEGF because it was more reproducible and consistent in estimating the activity of VEGF[86].

Hepatocyte growth factor: The hepatocyte growth factor (HGF) is a strong promoter of hepatocarcinogenesis through the activation of the HGF axis and its receptor MET[103]. Previous studies showed that high serum levels of HGF in patients with HCC negatively associated with OS and outcome[104-106]. In the recent SHARP study biomarkers analysis, patients treated with sorafenib experienced a decrease in a mean plasma level of HGF although; patients treated with placebo have mean HGF concentration increase[63]. Added to circulating stem-cell factor receptor (c-KIT) and angiopoietin 2 (Ang2) concentrations, HGF level was shown to be an independent factor of survival in patients with advanced HCC[63]. Low baseline HGF plasma level trends toward better OS (12.4 mo *vs* 6.3 mo, P = 0.073) and TTP in patients treated with sorafenib for HCC[63]. Noticeably, in contrast to plasma levels, tissue HGF expression carries low prognostic information[107]. Further investigations are needed to identify the role of HGF as a predictor of response to sorafenib in patients with advanced HCC.

Ang2: Ang2, one of the families of angiopoietins, is an angiogenic factor implicated in tumor angiogenesis stimulation and progression in human HCC[108]. Tumor overexpression of Ang2 was associated with vascular invasion, tumor size microvessel density level, poorly prognosis HCC[108,109] and poor differentiated tumor[110]. Preoperative presence of Ang2 in the hepatic vein was also associated with portal invasion and poor outcome in HCC resected patients[111]. In a small uncontrolled cohort of patients treated with sorafenib for advanced HCC, the authors reported that Ang2 could predict the outcome[112]. High Ang2 serum baseline level was associated with PFS but not with OS in HCC patients treated with sorafenib[112]. Llovet *et al*[63] confirmed the negative impact on prognosis of baseline high plasma level of Ang2 in HCC. In patients treated with sorafenib or placebo, median OS was significantly shorter in those with high baseline Ang2 plasma levels compared to those with low baseline concentrations (6.3 mo *vs* 14.1 mo, HR = 2.407; 95%CI: 1.9-3.03; P < 0.001). In the group of patients treated with sorafenib, no significant changes in median Ang2 plasma levels were observed during the treatment. However, concentration increase was reported in the group of patients treated with placebo[63]. Both patient groups treated with sorafenib or placebo that experienced an increase of Ang2 plasma levels during follow-up had shorter OS and TTP[63]. Ang2 seems, therefore, a prognostic factor of HCC aggressiveness but not an adequate predictive factor of sorafenib efficacy. Llovet *et al*[63] suggested that dosing Ang2 plasma levels during treatment with sorafenib could be an attractive option to monitor patients with advanced HCC.

Basic fibroblast growth factor: The basic fibroblast growth factor (bFGF) is one of the identified angiogenic factors with a potent stimulus for HCC growth[113]. Tumor overexpression of bFGF seems mainly implicated in HCC invasiveness than tumor neovascularization[114]. Moreover, a significant correlation between high preoperative serum bFGF level and larger tumor, venous invasion, advanced tumor staging and early recurrence was reported in resected HCC[115]. In the SHARP study,

no difference was observed concerning changes in mean bFGF plasma concentration between sorafenib and placebo in patients with advanced HCC[63].

Stem-cell factor receptor - KIT: The role of stem-cell factor receptor and its soluble forms has not been entirely elucidated in HCC. Soluble forms of KIT were fundamentally implicated in tumor-cell survival and proliferation[93]. Llovet *et al*[63] reported a trend to a positive impact of high baseline soluble c-KIT level on OS and TTP in patients treated with sorafenib. Sorafenib induced a significant decrease in mean plasma levels of soluble c-KIT, unlike the placebo that resulted in no changes in c-KIT concentration[63]. Likewise, following exposure to sunitinib, plasma levels of soluble c-KIT decreased significantly in patients with renal cell carcinoma[95], breast cancer[101] and HCC[91-93]. SHARP biomarker analysis showed a nonsignificant trend of soluble c-KIT in predicting sorafenib response in patients with advanced HCC. In the sorafenib cohort, patients with high baseline soluble c-KIT level showed better median OS and TTP compared to those with low soluble c-KIT level but without reaching significance (10.4 mo *vs* 9.4 mo, P = 0.081 and 6.7 mo *vs* 4.1 mo, P = 0.052; respectively)[63]. In a phase Ⅱ study, Zhu *et al*[91] reported that soluble KIT plasma levels decrease following 14 d of sunitinib treatment in patients with advanced HCC and correlated with better PFS and OS. Similarly, improvement of TTP and trend towards better OS were reported when soluble KIT plasma level decreased from baseline following sunitinib in patients with HCC, metastatic breast cancer and neuroendocrine tumor[93,95,101]. Nowadays, the role of soluble c-KIT in HCC pathogenesis remains unclear since the expression of this protein kinase in HCC tissue appears to be anecdotal[116].

Insulin growth factors: The insulin growth factors (IGF) signaling pathway, including its ligand, IGF-1, and IGF-2, plays a crucial role in carcinogenesis of various tumors[117,118]. In patients with HCC, independently to the tumor stage, low baseline IGF-1 plasma level correlated with poorer OS[87]. In a small cohort of patients with advanced HCC receiving first-line antiangiogenic treatment associated with metronomic chemotherapy, serum levels of IGF-1 could predict treatment efficacy in this population. Indeed, high baseline IGF-1 serum levels before treatment correlate with better OS, PFS and disease control rate[87]. Moreover, high baseline IGF-2 plasma levels associated with a better OS in the placebo group enrolled in the SHARP trial[63]. In this large phase Ⅲ controlled trial, the IGF-2 failed to predict response to sorafenib in patients with advanced HCC[63] confirming previous results observed with other antiangiogenic agents[119].

Circulating endothelial cells and circulating endothelial cell progenitors: In preclinical models, levels of circulating endothelial cells (CEC) and bone-marrow-derived CEC progenitors (CECP) were shown to be potential surrogate markers of angiogenesis[120,121]. High circulating level of CECP in patients with HCC correlates with advanced disease[122]. Previous studies reported levels of CEC and CECP decrease and return to normal values following antiangiogenic therapy in cases of complete remission[123]. Willett *et al*[124] showed that high doses of bevacizumab induce an increase of viable CEC and CECP percentage in a small cohort of patient with rectal cancer. Bevacizumab treatment induced in patients with advanced HCC, an early increase of viable CEC levels that correlated with objective response[98]. In patients with imatinib-resistant gastrointestinal stromal tumor, sunitinib induced early, but not subsequent increase of CEC blood levels that seemed to be correlating with clinical benefit[125]. Otherwise, sunitinib was shown to cause a decrease of CECP level in patients with advanced HCC[91]. Shao *et al*[126] showed that high baseline CECP level, but not CEC level, was associated with poor OS in patients treated with sorafenib combined with metronomic chemotherapy. The value of CEC and CECP levels as biomarkers of angiogenesis and antiangiogenic therapies in HCC needs further prospective analysis. In fact, methods and techniques of measurement were inconsistent, and unreliable results were reported depending on the type of study (clinical or preclinical studies), cancer types, and antiangiogenic agents[98,115,116,121].

In summary, none of the above biomarkers is validated to predict response to sorafenib in patient with advanced HCC. Except the SHARP biomarkers analysis study, the majority of available data was reported from no control arm retrospective studies. Validation through further large, controlled randomized trials are required to confirm the predictive value of such predictive biomarkers so to be integrated with clinical use. Moreover, techniques used to assess drug-induced variation in circulating factors should be standardized for reliable interpretation. An important issue should also be questioned of whether the presence or change in circulating biomarkers could discriminate between treatment benefit and tumor resistance or escape.

Tissue biomarkers

In addition to tissue prognosis markers obtained from tumor samples, some studies tried to identify predictive factors of response and outcome following anticancer agents. Table 7 summarizes studies evaluating tissue biomarkers used as prognostic and predictive of HCC. Abou-Alfa *et al*[127] evaluated the impact of tumor expression of phosphorylated extracellular signal-regulated kinase (pERK) and outcome in patients treated with sorafenib for advanced HCC. A high pretreatment tumor level of pERK correlated with TTP, but the survival impact was not analyzed. Tumor-cell expression and staining levels of pERK using immunohistochemistry analysis were performed in 33 patients. Patients with high pretreatment tumor-cell pERK expression had better TTP compared to those low staining intensity. The

Table 7 Prognostic and predictive value of tissue biomarkers evaluated in hepatocellular carcinoma

Ref.	Marker	Patient (*n*)	Origin of specimen	Method assay	Quantification	Marker level	Clinical impact
Mitsuhashi *et al*[108]	Ang2	46	Resected specimens	RT-PCR and IHC	Quantitative	High tumor Ang2/1 ratio	Tumor portal vein invasion Large tumor Increase MVD Poor OS
Zhang *et al*[109]		38	Resected specimens	RT-PCR	No	High tumor Ang2/1 ratio	Large tumor Portal vein invasion Metastasis
Torimura *et al*[110]		59	Resected specimens (19) and Biopsy (40)	RT-PCR and IHC	Semi-quantitative	High tumor Ang2	Poor differentiated tumor
Abou-Alfa *et al*[127]	pERK	33	Biopsy before sorafenib	IHC	Semi-quantitative	High tumor pERK	Better TTP
Ozenne *et al*[128]		20	Biopsy before sorafenib	IHC	Semi-quantitative	High tumor pERK	No impact
Hagiwara *et al*[131]	JNK	39	Biopsy before sorafenib	IHC and Western Blot	Quantitative	High JNK tumor	Lower ORR Poorer TTP Poorer OS
Peng *et al*[134]	pVEGFR-2	35	Resected specimen before sorafenib	RT-PCR and IHC	Semi-quantitative	Low tumor expression	Poorer OS
Poon *et al*[84]	VEGF	60	Resected specimen	IHC and ELISA	Semi-quantitative	High tumor expression	Advanced HCC stage

Ang2: Angiopoietin 2; ELISA: Enzyme-linked immunoadsorbent assay; IHC: Immunohistochemistry; JNK: C-Jun N-Terminal Kinase; MVD: Microvessel density; ORR: Objective response rate; OS: Overall survival; pERK: Phosphorylated extracellular signal regulated kinase; pVEGFR: Phosphorylated vascular endothelial growth factors receptor; RT-PCR: Real-time polymerase chain reaction; TTP: Time to progression.

authors speculated that tissue expression of pERK could be predictive of response to sorafenib since tumors with higher levels of pERK were associated with more sensitive, or responsive, to sorafenib[127]. Our immunohistochemistry analysis did not confirm these findings[128]. Indeed, immunophenotypical markers (including pERK, VEGF, CD34, CK19, and STAT3) were evaluated in 21 patients treated with sorafenib for advanced HCC. None of these tissue markers was predictive of survival in our population[128]. These inconsistent results could be explained by the significant variability of detection of ERK expression by immunohistochemistry between samples obtained from biopsies compared to their subsequent resected HCC specimens[129] and the potential for rapid dephosphorylation and variable time of tissue fixation[130].

Recently, a Japanese group found in patients treated with sorafenib for advanced HCC, a negative impact of tumor expression of phospho-c-Jun on outcome[131]. Tumor expression of phosphor-c-Jun was associated with low tumor response rate, shorter TTP and OS[131]. These data need further validation since limited samples were evaluated.

Otherwise, previous analysis showed that VEGF expression in HCC tumor was associated with aggressive disease and worse outcome[132,133]. Peng *et al*[134] showed that tumor expression of VEGFR (phosphorylated VEGFR-1 and VEGFR-2) could affect the outcome of patients treated with sorafenib for advanced HCC[134]. Using immunohistochemistry analyzes, low pVEGFR-1 and pVEGFR-2 expressions in previously resected HCC specimens; a subsequent treatment with sorafenib was associated with worse outcome and poorer OS. The authors postulated that high autocrine VEGF signaling activity in tumor tissue could be predictive of response and outcome in patients treated with sorafenib[134]. These results could be hampered somewhat by the retrospective feature of the analysis, the small number of patients included and the low feasibility in clinical practice.

Furthermore, overexpression of "stemness"-related proteins (including c-KIT, K19, and CD34) was shown to be associated with aggressive HCC and poor prognosis[135-138]. Recently, the stem-cell factor, a ligand of c-KIT, was shown to be an independent prognostic factor for HCC after resection[139]. In patients with low tumor expression of stem-cell factor, the median time to recurrence was 24 mo compared to 12 mo in patients with overexpression > 85% of the marker[139].

Microvessel density (MVD) was another tissue biomarker proposed to predict response to antiangiogenic agents. Willett *et al*[124] observed a decrease of tumor MVD following antiangiogenic therapies in rectal cancers and this parameter was suggested as predictive of clinical benefit. However, inconsistent results were reported in an exploratory analysis of a large pivotal trial evaluating the addition of bevacizumab to chemotherapy in patients with metastatic colorectal cancer[140]. The tumor MVD did not predict the survival benefit in this large trial[140]. Noticeably, measurement methods of MVD were not standardized explaining partially the inconsistent results[140]. MVD analysis of HCC tumor tissue was shown to have only prognostic value[141]. The feasibility of tumor MVD expression was very limited in clinical practice hampering its use in predicting response to antiangiogenic agents for HCC.

Some tissue markers of response were evaluated in HCC using other antiangiogenic agents. Tivantinib, a selective MET inhibitor, was evaluated in a second line setting through a randomized, placebo-controlled

phase Ⅱ trial in patients with advanced HCC[142]. In this study, tumor expression of MET influenced treatment benefit. Patients with tumor overexpression of MET clearly benefit from tivantinib treatment. High-MET tumor expression was associated with longer TTP on tivantinib compared to placebo (2.7 mo *vs* 1.4 mo, HR = 0.43, 95%CI: 0.19-0.97; *P* = 0.03) and OS (7.2 mo *vs* 3.8 mo, HR = 0.38, 95%CI: 0.18-0.81; *P* = 0.01). Interestingly, tivantinib did not show any benefit when tumor expression of MET was low[142].

Nowadays, no tissues biomarkers can identify patients who might respond to sorafenib. Tumor analysis data were/was unavailable in large clinical trials, probably because of lack of tumor samples biopsies since HCC diagnosis was frequently made according to imaging features[143,144].

IMAGING FEATURES AND FUNCTIONAL IMAGING

The clinical benefit of sorafenib with OS gain in patients with advanced HCC contrasted largely with a low objective response rate noted in this population. The low response rates could be considered as a sign of lack of antitumor activity in early phases of clinical trials but were favorably balanced by sustained tumor stabilization and small numbers of tumor progression in the waterfall plot activity. Fortunately, the decision to proceed with phase Ⅲ trials was not hampered by the apparent lack of tumor response.

Which response criteria to apply?

The conventional RECIST criteria[23,24] usually used for tumor response evaluation of conventional chemotherapy appear clearly inappropriate to evaluate the response to sorafenib in patients with advanced HCC. Major features were reported following antiangiogenic agents consisting of decreased tumor vascularization[145] and density[146] on computer tomography (CT) scans. The modified RECIST (mRECIST) criteria are a new assessment method proposed by Lencioni and Llovet[145] to overcome the limitations of RECIST criteria. They include vascularization and tumor arterial enhancement changes of the target lesion on CT. Other new criteria including European Association for the Study of the Liver (EASL) criteria and Choi criteria, that evaluated tumor density changes, were also proposed to evaluate tumor response to sorafenib in patients with HCC[100,146-148]. A representative case of discrepancies between these criteria is shown in Figure 1. Several studies used CT-scan evaluation to predict early response to sorafenib and to adjust treatment strategy according to the potential clinical benefit[100,147,149].

Edeline *et al*[147] showed in patients treated with sorafenib for advanced HCC that overall response rate was higher when mRECIST criteria were applied compared to RECIST criteria (22.7% *vs* 1.9%). Interestingly, tumor response assessment according to mRECIST criteria, reclassified 22.6% of patients as responders while they were initially categorized as having stable disease by RECIST criteria[147]. Our group found consistent results when alternative radiological criteria to RECIST were applied[100]. We evaluated early tumor response in 64 patients with advanced HCC treated with sorafenib using RECIST, mRECIST, Choi and EASL criteria[100]. These new criteria identified a higher objective response rate compared to the conventional RECIST criteria (varying from 51% for Choi to 28% and 28% for mRECIST and EASL respectively; compared to only 3% for RECIST criteria). Responder patients according to Choi criteria at the first tumor assessment had better OS compared to non-responders (22.4 mo *vs* 10.6 mo, 95%CI: 0.15-086; *P* = 0.097)[100].

Further evaluations of these new criteria in comparison to RECIST criteria are needed in prospective clinical trials evaluating sorafenib or other antiangiogenic agents for advanced HCC.

In summary, we believe that, combining early reduction of AFP levels following sorafenib initiation with new radiological criteria could be helpful in detecting patients who might benefit from antiangiogenic treatment and to propose better tailor-made strategy management.

Functional imaging

Various functional imaging tools [including contrast-enhanced ultrasound, dynamic contrast-enhanced magnetic resonance imaging (MRI) and dynamic contrast-enhanced CT and positron emission tomography (PET)] were proposed to evaluate the antiangiogenic effects[150] (Table 8). Functional imaging approaches consist of infusion of intravenous contrast agent that enhances vascular and tumor structures and the acquisition of sequential images before, during, and after injection.

Dynamic contrast-enhanced ultrasound

Some small cohort studies evaluated the useful of dynamic contrast-enhanced ultrasound (DCE-US) to predict early tumor response to sorafenib in patients with advanced HCC[151-153]. In a Japanese prospective monocentric study, a total of 37 patients with advanced HCC treated with sorafenib were evaluated using DCE-US, before treatment and on days 7, 14 and 28 of treatment[152]. Significant changes in different US perfusion parameters between responders and non-responders (according to RECIST and mRECIST criteria) were observed at the prescheduled time of the follow-up. Correlation between reduction in tumor blood volume 7 d after treatment initiation and better PFS and OS was found. The authors suggest that DCE-US performed earlier could be useful to identify patients with advanced HCC, who may benefit from sorafenib[152]. Consistent results were obtained in an Italian prospective study that enrolled 28 patients treated with sorafenib and monitored with DCE-US at baseline, days 7, 15 and 30 of treatment[154]. Early decrease of tumor vascularity

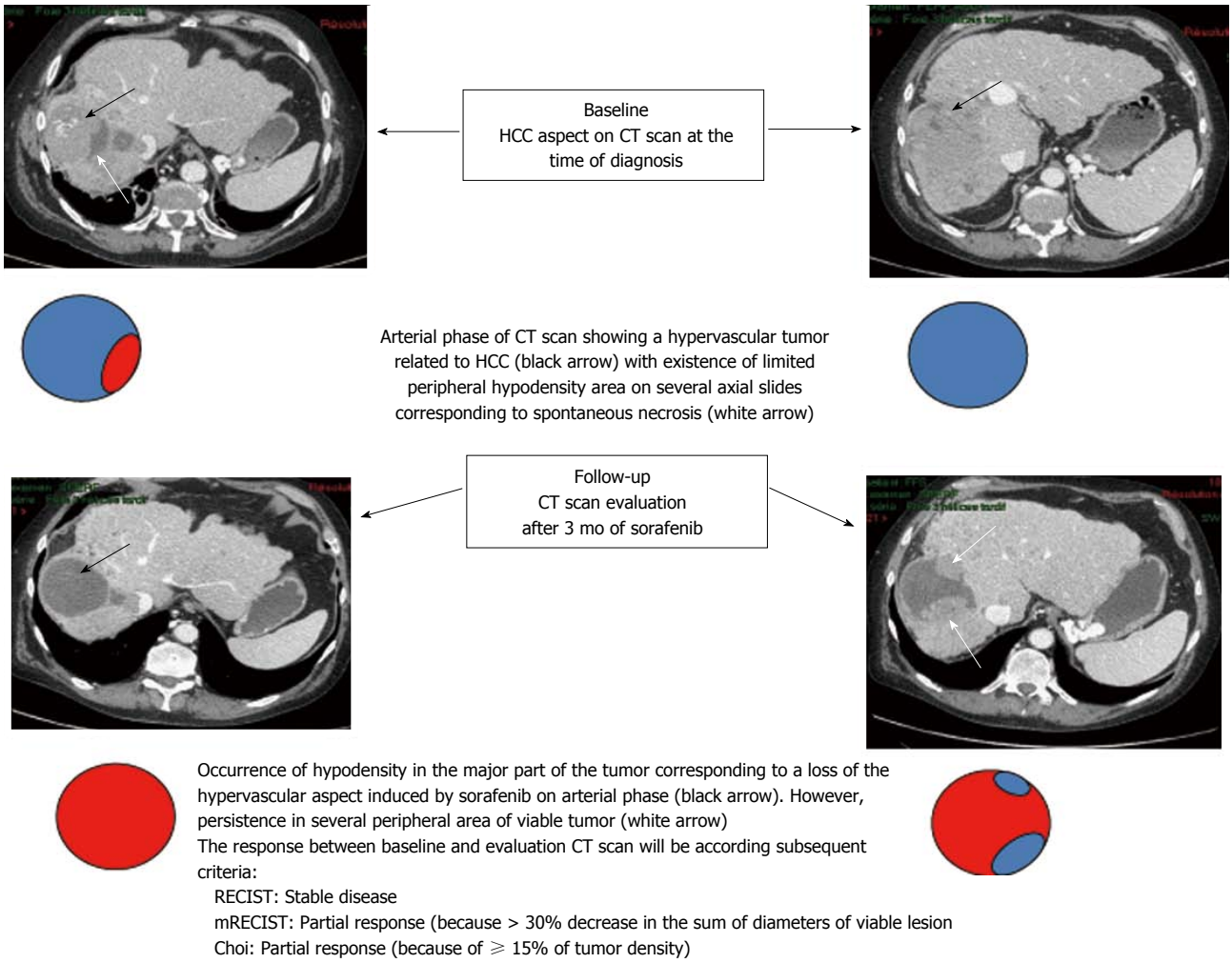

Figure 1 An illustrative case showing discrepancies between subsequent criteria used to assess tumor response in a patient treated with sorafenib for hepatocellular carcinoma. HCC: Hepatocellular carcinoma; CT: Computed tomography; RECIST: Response evaluation criteria in solid tumors; mRECIST: Modified RECIST.

Table 8 Value of functional imaging in patients with hepatocellular carcinoma treated with antiangiogenic agents

Ref.	Imaging tools	Patients (*n*)	Study design	Treatment	Imaging findings and clinical impact	Conclusion/comments
Sugimoto *et al*[152]	DCE-US	37	Prospective	Sorafenib	Tumor vascularity decreases and blood volume within seven days trends towards better PFS and OS	These studies enrolled small cohort of patients hampering adequate interpretation. However, DCE-US remains a promising noninvasive imaging, but operator dependent, to predict response in patients with HCC treated with sorafenib and larger cohort of patients should be evaluated
Zocco *et al*[153]		28	Prospective	Sorafenib	An early decrease in AUC and increase of median transit time was associated with better PFS and OS	
Zhu *et al*[91]	DCE-MRI	34	Prospective	Sunitinib	Decrease in vascular permeability was associated with better disease control	The decrease of vascular permeability induced by antiangiogenic agents seems to be a good predictive of tumor response and clinical benefit. These promising findings should be confirmed by largest cohort of patient
Hsu *et al*[156]		31	Prospective	Sorafenib + mT/U	A ⩾ 40% decrease in vascular permeability with 14 d was associated with better PFS and OS	
Lee *et al*[159]	FGD-PET	29	Retrospective	Sorafenib	SUV < 5.00 correlated with longer PFS and OS	Prospective studies are needed to evaluate the predictive value of the FDG-PET in HCC

AUC: Area under the time-intensity curve; DCE-US: Dynamic contrast-enhanced ultrasound; DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging; FGD-PET: 18F-fluorodeoxyglucose - positron-emission tomography; PFS: Progression-free survival; OS: Overall Survival; mT/U: Metronomic tegafur/uracil.

occurring during treatment was predictive of tumor response, better PFS and OS.

Dynamic contrast-enhanced magnetic resonance imaging

Dynamic contrast-enhanced magnetic resonance imaging has already been proposed to assess vascular disruption of antiangiogenic compounds in early clinical trials. However, this technique remains considerably more complex than conventional imaging and needs real expertise[155]. Using DEC-MRI, changes in tumor blood flow following VEGFR tyrosine kinase inhibitors were observed in patients with advanced HCC[91,156]. Significant decrease in vascular permeability (K^{trans}) and reverse reflux rate constant between the extracellular space and plasma (K_{ep}) were reported in patients with advanced HCC treated with sunitinib[91]. These changes were associated with better prognosis since the extent of decrease in K^{trans} was significantly greater in patients with partial response or stable disease compared to those with progressive disease or those who died early following sunitinib treatment[91]. DEC-MRI was also evaluated to predict response and benefit in 31 patients with advanced HCC treated with sorafenib plus metronomic tegafur/uracil[156]. In this study, K^{trans} before treatment was significantly higher in patients with partial response or stable disease compared to patients with progressive disease. Following 14 d of treatment, significant change in median K^{trans} was observed in responders compared to non-responder patients (-47.1% *vs* 9.6%; $P < 0.001$). The percentage of K^{trans} change following treatment was an independent predictor of tumor response, PFS, and OS. Better PFS, and OS was seen when a vascular response, defined as $\geqslant$ 40 decrease in K^{trans} at day 14 of treatment, was detected (29.1 wk *vs* 8.7 wk, $P = 0.033$ and 53.0 wk *vs* 14.9 wk, $P = 0.016$; respectively)[156].

Currently, the use of DEC-MRI is limited to clinical research and has not been extended to routine practice. Further studies combining cost-effectiveness are needed to define the place of this innovative tool as predictive of tumor response and clinical benefit with sorafenib in advanced HCC.

^{18}F-fluorodeoxyglucos-PET

Few studies evaluated the prognostic value of ^{18}F-fluorodeoxyglucose-PET (18-FDG-PET) in patients receiving antiangiogenic agents for advanced HCC[157,158]. In a small cohort study, Lee *et al*[159] found that the degree of FGD uptake correlates with outcome in Korean patients with advanced HCC treated with sorafenib. Patients who experienced pretreatment standardized uptake values (SUV) < 5.00 had better PFS and OS compared to those with SUV $\geqslant$ 5.00[159]. Undeniably, such findings should be verified by prospective evaluation in large cohort patients. Finally, no data are/is available regarding the prognostic or predictive value of ^{18}F-fluorocholine, a PET tracer of lipid metabolism, that is supposed to be more sensitive than ^{18}F-FDG for HCC detection[158], in patients receiving antiangiogenic drugs for HCC.

In summary, several studies with antiangiogenic agents have shown the need for additional criteria, beyond RECIST criteria, for early evaluation of antitumor activity and identification of patients who could benefit from these therapies. Furthermore, promising findings of the correlation between biomarkers and radiological response were shown in some studies, warranting further validation in larger clinical trials.

Measurement of tumor hypodensity, intratumor necrosis, and vascular parameters are the main criteria to be explored by dynamic functional imaging. These parameters are not already validated, but they represent prospective radiological investigations of primary interest for the assessment of antiangiogenic therapy effects beyond tumor size.

CONCLUSION

The sorafenib success story in advanced HCC raised new questions regarding the suitable approach to select patients who would likely benefit from treatment, ideally before its initiation. In routine practice, identifying predictive tools and biomarkers of response or early resistance seems to be an unmet need. Nowadays, no one of biomarkers the cited above biomarkers was validated in routine. AFP and some proangiogenic factors, such as VEGF and Ang2, seem to be promising prognostic and predictive biomarkers in HCC. However, there is probably no single ideal biomarker to predict response to antiangiogenic agents.

Controlled-arm prospective studies are required to improve the robustness of result interpretation. New endpoints are necessary for these biomarkers, such as monitoring angiogenesis, predicting early treatment response or even before starting therapy, defining optimum biological dose and identifying early resistance to antiangiogenic agents. Translational research using sequential tumor biopsy analysis while the patient is his own witness could probably be the most reliable method to identify robust biomarkers. Furthermore, advances in functional imaging techniques could allow evaluation of these molecules in real time, by assessing tumor density rather than tumor size. New tumor assessment criteria, particularly in cases of stable disease according to RECIST, should be identified and validated through large prospective cohort analysis. Finally, combining imaging response and efficient circulating biomarkers such AFP or proangiogenic factors (*e.g.,* VEGF or Ang2) could be a practical option and may be helpful to detect patients more likely to benefit from antiangiogenic treatment and to propose better tailor-made strategy management.

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REVIEW

Markers of bacterial translocation in end-stage liver disease

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Abstract

Bacterial translocation (BT) refers to the passage of viable bacteria or bacterial products from the intestinal lumen, through the intestinal epithelium, into the systemic circulation and extraintestinal locations. The three principal mechanisms that are thought to be involved in BT include bacterial overgrowth, disruption of the gut mucosal barrier and an impaired host defence. BT is commonly observed in liver cirrhosis and has been shown to play an important role in the pathogenesis of the complications of end stage liver disease, including infections as well as hepatic encephalopathy and hepatorenal syndrome. Due to the importance of BT in the natural history of cirrhosis, there is intense interest for the discovery of biomarkers of BT. To date, several such candidates have been proposed, which include bacterial DNA, soluble CD14, lipopolysaccharides endotoxin, lipopolysaccharide-binding protein, calprotectin and procalcitonin. Studies on the association of these markers with BT have demonstrated not only promising data but, oftentimes, contradictory results. As a consequence, currently, there is no optimal marker that may be used in clinical practice as a surrogate for the presence of BT.

Key words: End stage liver disease; Cirrhosis; Soluble CD14; Bacterial DNA; Lipopolysaccharides endotoxin; Procalcitonin; Bacterial translocation; Calprotectin; Biomarkers; Lipopolysaccharide-binding protein

Core tip: The exact mechanism behind bacterial translocation in patients with cirrhosis has not been fully elucidated. The discovery of reliable biomarkers for this phenomenon would be of significant clinical importance, as bacterial translocation is closely associated with the development of severe complications. Various molecules have been identified as candidates for serving as markers of bacterial translocation in this patient population. This mini-review attempts to summarize the most recent available data regarding the potential use of such markers as clinical and prognostic tools in the management of end-stage liver disease.

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INTRODUCTION

Bacterial infections are frequent complications of cirrhosis and have been associated with significantly increased mortality rate[1]. Spontaneous bacterial peritonitis (SBP), urinary tract infections, pneumonia and sepsis are the most common infections, with gram-negative and gram-positive bacteria being equally detected as the causative organisms. In particular, cirrhotic patients with gastrointestinal bleeding have higher risk for developing bacterial infections during hospitalization and, thus, antibiotic prophylaxis is recommended in this scenario[2,3].

Bacterial translocation (BT) refers to the entry of viable bacteria or their products into the regional lymph nodes, the systemic circulation, and possibly extraintestinal organs. The origin of such microorganisms is the enteric flora and translocation occurs *via* a defective mucosal barrier[4]. BT is considered the key step in the pathogenesis of SBP and bacteremia in cirrhotic patients, as well as a critical factor that triggers host immune responses and secretion of inflammatory mediators, which, ultimately, mediate the hemodynamic changes that are present in portal hypertension and cirrhosis[5]. The main three mechanisms involved in BT include bacterial overgrowth, physical disruption of the gut mucosal barrier and an impaired host defence (Figure 1)[6]. In the present article, we will briefly review the pathogenesis of BT and analyze the literature regarding surrogate markers for this condition.

Pathogenesis of BT

Small intestinal bacterial overgrowth (SIBO) is multifactorial and may be the result of defective gastric acid secretion and compromised small intestinal motility, as well as dysregulated mucosal and systemic immunity. The currently accepted criterion for the diagnosis of SIBO is the presence of > 10^5 colony-forming units/mL of coliform bacteria in aspirates from the proximal jejunum. Alternatively, breath tests have been used as sensitive and simpler tools for diagnosis of bacterial overgrowth, by measuring an increase in breath hydrogen or methane concentration, produced from intestinal bacterial fermentation after glucose or lactulose ingestion[7]. Experimental data has shown that cirrhotic rats with SIBO had a significantly higher rate of BT and slower intestinal transit than those without SIBO[8]. In clinical studies, the prevalence of SIBO in cirrhotic patients was found to be significantly higher than that in non-cirrhotic controls, and that it is related to the severity of liver disease[9]. Furthermore, the incidence of SIBO was higher in patients with a previous history of SBP than in SBP-naïve patients[10].

Structural and functional alterations of the gut mucosa that lead to increased intestinal permeability to bacteria and their products have been described in cirrhosis[11]. Bile secretions may also play a role in the prevention of BT by inhibiting bacterial overgrowth, exerting a trophic effect on intestinal mucosa and neutralizing endotoxin[12]. Increased intestinal permeability has been linked to the progression of liver disease and the complications of cirrhosis[13]. However, increased intestinal permeability cannot fully account for the pathophysiology of BT; moreover, it is not clear whether these structural changes are the cause or the result of BT[5].

The intestinal immune system is comprised of Peyer's patches, the mesenteric lymph nodes (MLNs) and a large number of cells distributed throughout the lamina propria and epithelium of the intestine[14,15]. Patients with cirrhosis exhibit systemic immune alterations that may promote the development of infections and BT. Advanced cirrhosis is associated with decrease in the cellular and humoral components of immune response, decreased activity of the reticuloendothelial system, decreased phagocytic capacity of Kupffer cells, as well as restricted recruitment of leucocytes in response to inflammatory stimuli due to portal hypertension-associated splanchnic hyperaemia[16-18]. The inflammatory response induced by BT, with the synthesis of cytokines, particularly tumor necrosis factor-alpha (TNF-α), interleukins and nitric oxide (NO) also increases intestinal barrier permeability, which, in turn, favours BT, thus creating a feedback in which BT promotes its own causative mechanisms[5].

Bacterial translocation is also associated with systemic complications and deterioration of the hyperdynamic circulation in cirrhosis (Figure 1). Human studies have shown that cirrhotic patients with increased levels of lipopolysaccharide binding protein (LBP), a marker of BT, are found to have a significant immune and haemodynamic derangement, which is ameliorated by norfloxacin administration, by causing selective intestinal decontamination and inhibiting BT[19]. Additionally, the presence of bacterial DNA (bDNA), another marker of BT, in patients with cirrhosis and ascites, has been correlated with aggravation of peripheral vasodilation and with worsening of intrahepatic endothelial dysfunction[20]. Kidney tissue in cirrhosis shows increased expression of the toll-like receptor 4 (TLR4), nuclear factor κB (NF-κB), and TNF-α molecules, which makes the renal system further susceptible to the effects of cirrhosis during BT, highlighting the fact that BT contributes to the development of hepatorenal syndrome (HRS)[21]. Moreover, it has been reported that the non- absorbable antibiotic rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites, by suppressing intestinal bacterial overgrowth and preventing BT[22]. It has been also suggested that cirrhotic patients may have compromised ability to upregulate sufficient dilatory forces [*i.e.*, endothelial nitric oxide synthase (NOS), inducible NOS, and heme oxygenase-1] to counterbalance the constrictive effect of endothelin-1 upon a secondary insult of endotoxemia. This is indicated by the presence of lipopolysaccharides (LPS) endotoxin during BT, and the net effect of this phenomenon is the establishment of increased intrahepatic resistance[23]. Endotoxemia may also be a trigger factor for variceal bleeding,

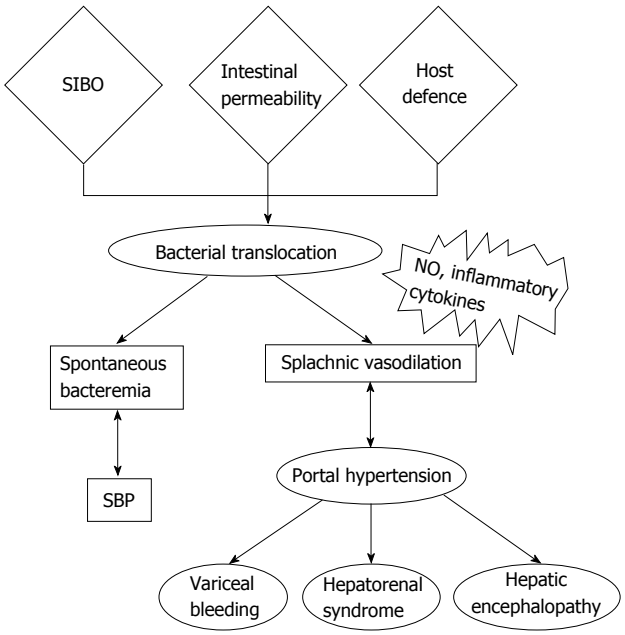

Figure 1 The main three mechanisms involved in bacterial translocation include intestinal bacterial overgrowth, increased intestinal permeability to bacteria and an impaired host defence. BT is considered the key step in the pathogenesis of bacteremia and SBP in cirrhotic patients, as well as a critical factor that triggers host immune responses and secretion of inflammatory mediators from macrophages, hepatocytes and other cells which, ultimately, mediate the hemodynamic changes that are present in portal hypertension and cirrhosis. BT contributes to the development of hepatorenal syndrome, and it may also be a trigger factor for variceal bleeding. Infection and the resulting systemic inflammatory response are considered important factors contributing to worsening hepatic encephalopathy in cirrhotic patients. SIBO: Small intestinal bacterial overgrowth; SBP: Spontaneous bacterial peritonitis; NO: Nitric oxide; BT: Bacterial translocation.

either by worsening liver function or causing an acute increase in portal hypertension[24]. Infection and the resulting systemic inflammatory response (SIRS) are considered important factors contributing to worsening hepatic encephalopathy (HE) in cirrhotic patients[25]. BT-associated inflammatory response may therefore have a role in the pathogenic mechanisms involved in HE. Consistent with this are the results from studies showing either improvement of minimal encephalopathy in cirrhotic patients receiving rifaximin, or the effectiveness of probiotics for secondary prophylaxis of HE, overall suggesting that BT plays an important role in the pathogenesis of HE[26].

Considering the eminent role of BT in the progression of liver disease and the subsequent complications of cirrhosis, it is not surprising that the elucidation of the underlying mechanisms as well as the discovery of possible markers of BT, that may be easily measured and have prognostic value for the severity of liver disease, has been at the center of attention of many research groups. In this review, we aimed to summarize the most prominent of the proposed markers of BT, such as bDNA, LPS, LBP, sCD14, calprotectin and procalcitonin (Table 1).

Bacterial DNA

The occurrence of bacterial-derived material in extraintestinal locations has been long recognized in clinical and experimental cirrhosis. At the time of liver surgery, one third of cirrhotic patients with Child-Pugh stage C demonstrate infected MLNs; this percentage is reduced to the level of non-cirrhotic patients after selective intestinal decontamination[27]. In contrast, most of BT episodes in cirrhotics remain undetected as its diagnosis relies on blood or ascitic fluid cultures, which are more often negative than positive. This may be the result of bacterial opsonization which renders bacteria nonviable in routine cultures. Despite the process of opsonization, bacterial components might remain in biological fluids and could be detected with more sensitive analytical methods. Taken together, these findings emphasize the need for the discovery of novel and reliable markers with high sensitivity and specificity for the diagnosis of BT[28].

The application of bDNA as a marker of BT was initially shown in animal models of experimental cirrhosis. Specifically, in cirrhotic rats the presence of bDNA of a certain bacterial species in blood, ascites or pleuritic fluid was always associated with its simultaneous presence in MLNs. Moreover, the presence of bDNA was associated with marked inflammatory responses[29]. Importantly, these findings occurred independently of the blood culture status (positive or negative).

Several studies in humans have now tested the validity of molecular detection of bDNA as a surrogate marker of BT. Using a polymerase chain reaction (PCR) - based method, Such *et al*[28] reported that bDNA in serum and ascitic fluid was present in 32% of cirrhotic patients with culture negative ascites, and that this likely represented episodes of single clone translocation and systemic seeding. *E. coli* was the most frequently identified microorganism, while *S. aureus* was responsible less frequently. The same group showed that bacteria persist in the blood of cirrhotic patients during variable periods of time after the completion of therapeutic paracentesis, therefore suggesting that this phenomenon is related to the existence of repeated episodes of BT from the intestinal lumen. The presence of identical sequences of nucleotides in all bDNA PCR fragments detected in every patient, strongly supports the existence of the repeated episodes of BT being caused by the same bacteria specie[30]. The presence of bDNA in patients with decompensated cirrhosis has also been associated with marked activation of peritoneal macrophages, as evidenced by NO synthesizing ability along with enhanced interferon-γ, TNF-α, interleukin (IL)-2, and IL-12 production[31]. Serum and ascitic fluid TNF-α levels were significantly higher in patients with bDNA compared to those without this marker on admission. Additionally, the relative risk of death, HRS and SBP was higher in patients with bDNA[32]. A sub-group of patients with translocation of Gram-positive microorganisms showed increased proinflammatory cytokines unrelated to endotoxin[33]. Furthermore, the presence of bDNA in patients with cirrhosis during an episode of ascites was shown to be an indicator of poor prognosis. When

Table 1 Biological markers for assessment of bacterial translocation in liver cirrhosis

	Origin	Comments	Ref.
Bacterial DNA	Bacteria	Pros: Long half-life, association with cytokine production, hemodynamic changes and prognosis	Bellot *et al*[20], Francés *et al*[31], El-Naggar *et al*[32], González-Navajas *et al*[33], Zapater *et al*[34], Jun *et al*[35], Fagan *et al*[36] Rincón *et al*[39], Sersté *et al*[40], Feng *et al*[41], Fujita *et al*[42], Vlachogiannakos *et al*[43], Mortensen *et al*[44], Mortensen *et al*[45], Appenrodt *et al*[46]
		Cons: Variable rates of detection (maybe depending on methodology used), lower prevalence in outpatients	
LPS	Gram (-) bacteria	Pros: Correlation with TNF-α, stage of cirrhosis, prognostic value for severity of liver damage	Hanck *et al*[52], Lin *et al*[54], Chan *et al*[55]
		Cons: Short half-life, variation of detection rates	Bellot *et al*[5], Fukui *et al*[53], Kaser *et al*[56], Stadlbauer *et al*[57]
LBP	Acute phase protein triggered by LPS	Pros: Long half-life, correlation with TNF-RI, TNF-α, IL-6, and hyperdynamic circulation	Albillos *et al*[19], Albillos *et al*[59]
		Cons: Low detection rates, elevated in systemic infection from Gram (-) bacteria	Albillos *et al*[19], Albillos *et al*[59]
sCD14	Monocytes, liver	Pros: Prognostic marker of disease progression in HBV/HCV/HIV, NAFLD and alcoholic liver disease, correlation with liver fibrosis, easily measured	Landmann *et al*[61], Tuomisto *et al*[62], Sandler *et al*[63], Balagopal *et al*[64], French *et al*[65], Ogawa *et al*[66], Campos *et al*[67]
Calprotectin	Neutrophils	Pros: Easily measured, fecal levels associated with stage of liver disease, SBP and HE, ratio of ascites calprotectin/total protein may be better	Gundling *et al*[77], Lutz *et al*[81]
		Cons: Plasma levels do no distinguish cirrhotic patients from healthy controls, weak association with alcoholic liver disease	Homann *et al*[75], Homann *et al*[76], Montalto *et al*[78]
Procalcitonin	Neutrophils, liver, thyroid	Pros: Ascitic levels may differentiate between cirrhotic subgroups	Attar *et al*[97]
		Cons: No correlation with HE, conflicting results depending on etiology of liver disease	Spahr *et al*[92], Elefsiniotis *et al*[94], Rahimkhani *et al*[95], Villarreal *et al*[96]
ANCAs (IgA)	Neutrophils	Pros: Associated with ascites and advanced cirrhosis, predicts time to the first infectious complication	Papp *et al*[99]
		Cons: Single study	

LPS: Lipopolysaccharides; HE: Hepatic encephalopathy; SBP: Spontaneous bacterial peritonitis; LBP: Lipopolysaccharide binding protein; ANCA: Anti-neutrophil cytoplasmic antibodies; TNF-α: Tumor necrosis factor-alpha; IL: Interleukin; HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus; NAFLD: Non-alcoholic fatty liver disease.

considering only patients with MELD score < 15, mortality was significantly higher in those positive for bDNA. In this study, SBP developed independently of the bDNA status at admission[34]. The presence of bDNA was also associated with peripheral vasodilation and deterioration of intrahepatic endothelial function[20]. Another study highlighted the strong correlation between SIBO and the presence of bDNA in the peripheral blood of patients with cirrhosis[35]. Moreover, the high bDNA detection frequency was recently confirmed as it was shown that it was detected in ascitic fluid from 23 of 25 patients with culture-negative, non-neutrocytic ascites. Again, bDNA levels were a poor prognostic factor for a 6-mo clinical outcome. High bDNA burden was also associated with reduced major histocompatibility complex class Ⅱ expression on macrophages isolated from ascites[36-38].

Studies on the importance of bDNA detection in cirrhotics have occasionally created contradictory results. bDNA was identified by gel electrophoresis of a multiplex PCR-based product which amplified selected prokaryotic nucleic acids and was detected in 5/5 culture-positive neutrocytic, 1/6 culture-negative neutrocytic and 8/56 culture-negative non-neutrocytic samples. Three-month mortality was increased in the presence of ascitic bDNA only for patients with a MELD score > 15[39]. Contrary to hospitalized patients, bDNA was rarely detected in ascitic fluid and serum of asymptomatic outpatients with cirrhosis and non-neutrocytic ascites, after repeated paracentesis[40]. This finding may account for the markedly low prevalence of SBP in cirrhotic outpatients, when compared with their hospitalized counterparts. In another study, bacterial-specific 16S ribosomal RNA was not detected in blood samples from systemic or splanchnic circulation of cirrhotic patients on days 0 and 29 after rifaximin administration. In this study, plasma bDNA concentration did not correlate with systemic hemodynamic parameters[43]. Furthermore, bDNA did not correlate with markers of inflammation, such as C-reactive protein, TNF-α, IL-6 and IL-8. Additionally, it did not accurately predict the presence of SBP[44]. bDNA was also found to be largely unrelated to a panel of markers of inflammation and without association with portal pressure in patients with cirrhosis undergoing transjugular intrahepatic portosystemic shunt insertion[45]. No correlation between detection of bDNA in ascites and SBP was found[46]. In another study, administration of a probiotic mixture improved the hepatic and systemic haemodynamics in cirrhotic patients, but these changes were not related to the detection of bDNA[39].

In all, these contradictory results may be accounted for by differences in the tested populations and, also, in the specific methodologies used for the detection of bDNA[41,42]. These discrepancies obviate the need

for larger studies which will include well-characterized patient subpopulations, including chronic hepatitis, non-alcoholic fatty liver disease (NAFLD), as well as compensated and decompensated cirrhosis. Moreover, standardisation of methodology is required in order to determine the applicability of bDNA usage in clinical practice as a surrogate marker for BT. Finally, testing of larger cohorts of patients may define the significance of detectable bDNA as a prognostic tool for systemic responses and survival in cirrhosis.

LPS-LBP-sCD14

LPS or endotoxin is a lipopolysaccharide, which is part of the outer membrane of Gram-negative bacteria. In circulation, it is recognized by LBP. The LPS-LBP complex binds to membrane CD14 (mCD14) on myeloid cells or to circulating CD14 (soluble CD14, sCD14)[47,48] and promotes a cascade of inflammatory responses *via* myeloid differentiation-2/TLR4 activation of NF-κB[49,50]. Kupffer cells, which are specialized macrophages located in the liver, are activated when exposed to LPS and can produce a spectrum of cytokines and reactive oxygen intermediates, including the proinflammatory cytokine TNF-α. Thus, LPS is a potent stimulator of Kupffer cell TNF-α production, and this pathway has been implicated in the pathogenesis of many types of liver injury. It has also been proposed that through this mechanism hepatic stellate cells are activated towards the production of inflammatory and adhesion molecules, thus inflicting liver damage[51].

Due to its aforementioned properties, LPS was one of the first molecules that was proposed as a marker of BT. The working hypothesis is that its presence in the sera of cirrhotic patients directly indicates endotoxemia from Gram negative bacteria. In alcoholic cirrhosis, LPS has been found to correlate with TNF-α levels and with the stage of liver disease (Child-Pugh score); thus, it has been suggested that LPS may be a key player in the progression of alcoholic liver disease[52]. Levels of LPS in plasma of patients with alcoholic cirrhosis were higher than in non-alcoholics, pointing to a critical role for alcohol consumption in the development of endotoxemia and liver damage[53]. Taking into consideration that LPS plasma levels of cirrhotic patients are elevated compared to chronic hepatitis and the reported correlation with the stage of cirrhosis, it is probable that LPS may serve as a prognostic factor of disease severity[54] and short-term survival of cirrhotic patients[55]. It should be noted, however, that not all studies have demonstrated a positive correlation between serum LPS and stage of cirrhosis[53,56]. Therefore, LPS has not been clearly established as a reliable marker of BT[57]. This may be due to the short half-life of LPS or to the interference of various factors with the detection of LPS[5].

LBP is a 65 kDa, acute phase protein that is predominantly produced in the liver by hepatocytes. Bacterial LPS triggers the production of LBP and the peripheral levels of LBP are significantly elevated in the setting of bacterial presence. LBP is known to specifically bind and transfer bacterial LPS, and the LPS-LBP complex binds mCD14 on myeloid cells or to sCD14[58]. Consequently, several studies have tested the validity of the expression of LBP as an indicator of a systemic response to LPS, and, thus, as an indirect marker of BT. Albillos *et al*[19] demonstrated that BT, expressed by elevated plasma levels of LBP, leads to derangement of patients with decompensated cirrhosis. Cirrhotic patients with high LBP levels had enhanced expression of sCD14 and sTNF-RI as well as elevated circulating levels of TNF-α and IL-6. Furthermore, an intense hyperdynamic circulatory state was described in this population. Interestingly, LPS was detectable only in one third of the patients with high LBP. The latter observation strengthens the suggestion that LPS cannot easily serve as a marker of BT, due to its short half-life, so transient episodes of bacteremia may remain undiagnosed. On the other hand, the subsequent expression of LBP after endotoxemia is detectable for a much longer period. Moreover, a study performed by the same group suggested that patients with high LBP circulating levels have increased susceptibility to infections[59]. Different studies have proposed that increased levels of LBP in cirrhosis are the result of chronic endotoxemia and that also no difference occurs in the expression of LBP between alcoholic and non-alcoholic cirrhosis. Kaser *et al*[56] reported a strong correlation between the two binding proteins of LPS, LBP and sCD14. Although its longer half-life makes LBP a more attractive marker of BT than LPS, its use has certain drawbacks. First, systemic LBP elevation exists only in response to Gram-negative bacteria, and, second, it may not only be present in BT, but also in systemic infection resulting in SIRS (systemic inflammatory response syndrome)[60].

Recently, the target molecule of the complex LPS-LBP, CD14 was also proposed as a surrogate marker of BT. mCD14 is expressed mainly by macrophages and at a lesser extent by neutrophils and dendritic cells. sCD14 is secreted by hepatocytes and monocytes. The expression of sCD14 is upregulated as a result of the presence of LPS. CD14 acts as a co-receptor along with TLR-4 for the detection of bacterial LPS[61]. LPS induces the CD14/TLR4 complex endocytosis in human monocytes and macrophages and the consequent NF-κB activation. Tuomisto *et al*[62] showed a significant correlation between the levels of bDNA in the liver and the local expression of CD14 in alcoholic liver disease. These findings emphasize the role of CD14 as a marker of BT. Sandler *et al*[63] proposed that sCD14 may not only be a marker of BT, but also a prognostic marker of disease progression in HBV and HCV infection. Specifically, sCD14 in patients with severe fibrosis was highly elevated not only in peripheral blood, but also within the hepatic parenchyma, measured by CD14 (+) hepatic cells. Regardless of the etiology of cirrhosis, microbial translocation as identified by the presence of sCD14, is believed to play a key role in the progression of liver disease[56]. Studies concerning cirrhosis in HIV-infected patients support this hypothesis[64]. French *et al*[65] provided evidence

that in HIV/hepatitis C virus (HCV)-coinfection, levels of sCD14 and IL-6 were mostly elevated in patient with disease progression than in non-progressors. Studies in patients with NAFLD reached similar results, suggesting a positive correlation between serum sCD14, hepatic CD14 expression and liver inflammation[66]. Attention has also been drawn towards polymorphisms of the promoter region of the *CD14* gene, shedding light to the role of CD14/-159TT genotype in the progression of liver injury in alcoholic liver disease[67]. Taking into account the aforementioned studies, it appears that sCD14 may represent a promising marker of BT as it can easily be measured in circulation and correlates with progression of liver disease.

Calprotectin - procalcitonin - anti-neutrophil cytoplasmic antibodies

Calprotectin is a calcium and zinc binding protein with a molecular weight of 36 kDa[68]. It has been estimated that it may account for more than 60% of the soluble cytosolic proteins in human neutrophil granulocytes[69]. Moreover, it was shown that calprotectin measurements in fecal samples not only correlate with the degree of neutrophil migration in the gastrointestinal mucosa[70], but also serve as reliable surrogate marker of intestinal inflammation[71]. As a result, fecal calprotectin has been studied in depth in gastrointestinal disorders, and has assumed an important role in monitoring the activity and response to treatment in patients with inflammatory bowel disease[72,73].

The pathogenesis of BT in patients with cirrhosis has been associated with alterations in gut mucosal immune responses and intestinal permeability. In addition, neutrophil infiltrates are detected in the gastrointestinal mucosa of cirrhotics. Consequently, calprotectin has been investigated as a possible diagnostic marker for the existence and natural history of SBP and HE. Initial studies conducted by a Danish research group, focused on the possible prognostic significance of calprotectin levels in the plasma and ascitic fluid samples from patients with end-stage liver disease. The authors did not find a significant difference between healthy controls and patients with cirrhosis (irrespectively if liver disease was compensated or decompensated), a finding that was confirmed in additional studies[74,75]. On the other hand, they reported that high plasma calprotectin levels were an indicator of poor survival in alcohol-related cirrhosis[74]. The most important finding, however, regarding the role of calprotectin in relation to BT, was that during follow up of the patients higher calprotectin levels were an independent predictor of recurrent bacterial infections[76].

Arguably the most important study regarding the role of fecal calprotectin in the diagnosis of BT complications, is the one conducted by Gundling *et al*[77]. They investigated the relationship between fecal calprotectin levels and the onset and course of SBP and HE. They confirmed that patients with cirrhosis had significantly elevated fecal calprotectin levels when compared to healthy controls. Moreover, this increase correlated with the severity of liver disease (assessed by Child-Pugh and MELD scores). Even more significantly, higher calprotectin values were associated with advanced stages of HE, the presence of SBP, as well as extraintestinal infections. Finally, calprotectin strongly correlated with serum ammonia levels[77]. The aforementioned observations reinforced the hypothesis that fecal calprotectin may be a reliable surrogate marker for BT and provide important assistance in the diagnosis and clinical management of patients with decompensated liver disease. It should be noted that the finding of a possible selective calprotectin upregulation in alcoholic liver disease that was reported in initial studies, was not confirmed in a longitudinal study of active alcoholics, where alcoholics and controls had similar fecal calprotectin measurements[78].

It was recently reported that ascitic calprotectin may be utilized (with the help of a point-of-care assay) to reliably predict an elevated polymorphonuclear count (> 250) in ascitic fluid, allowing for faster diagnosis of SBP[79]. Another study by Alempijević *et al*[80] focusing exclusively on HE confirmed that fecal calprotectin levels were positively correlated with HE grading according to the West-Haven grouping criteria, although it did not show a correlation with serum ammonia levels as Gundling *et al*[77] did. Finally, the ratio of ascites calprotectin to total protein was proposed as a better marker than ascitic fluid calprotectin alone for use in the diagnosis and prognosis of SBP. The authors report satisfactory sensitivity and specificity for this new marker, as well as a statistically significant correlation of higher values with poor 30-d survival[81].

In all, calprotectin remains a promising surrogate marker for BT in cirrhosis. It demonstrates many advantages, especially in its fecal measurement, as it is a non-invasive, quick and relatively easy to perform assay, with proven clinical value in other disease states[82].

Procalcitonin (PCT) is a 116 amino acid propeptide of calcitonin. It has been established as a valuable biomarker for the diagnosis and monitoring of bacterial infections to the point that is being used as a guide for antibiotic use[83]. Concerning advanced liver disease, PCT has been studied for the past 15 years regarding its potential for the diagnosis of SBP in decompensated patients and subsequently for its utility as an indirect marker of BT. As the liver is believed to be a key source of PCT, there were initially concerns that hepatic impairment may result in downregulated serum PCT levels. Although, this was not proven to be the case[84], and early reports were encouraging about the use of PCT in the diagnosis of SBP, its potential role remains currently unclear as several studies provided conflicting results[85-93]. A solution has been proposed with the use of an ultra-sensitive PCT assay[87], and possible explanations for the discrepancies noted between studies include higher baseline levels in patient with alcoholic[94] or specific viral-related[95] causes of cirrhosis and the

presence of other bacterial infections[96]. Furthermore, it was reported that PCT levels in the ascitic fluid, but not in serum may differentiate between different cirrhotic subgroups, reflecting a possible localized role in the interplay between ascites and BT[97]. Interestingly, in contrast to calprotectin, PCT has not been found to correlate with the presence of HE, another possible important component of the BT phenotype in cirrhosis. It should be also noted, that in several of the PCT-related studies the role of CRP was assessed as well and it was usually found to have an inferior potential as a SBP marker when compared to PCT[91,98].

Finally, as part of the potential of established auto-immune and inflammatory markers of BT, a recent Hungarian study is worth mentioning. Therein, it was reported that the prevalence of anti-neutrophil cyto-plasmic antibodies (ANCA) of the IgA subtype was higher in patients with cirrhosis in comparison to patients with non-cirrhotic chronic liver disease or healthy controls. Moreover, the presence of IgA ANCA was associated with the presence of ascites and advanced cirrhosis. Even more significant was the finding that, during follow up, patients with IgA ANCA had not only a higher complication risk but also IgA ANCA positivity correlated with a shorter time to the first infectious complication[99]. As IgA antibodies are linked to the gut immune system, these results may reflect a component in the pathogenesis of BT involving alterations in the local immune system and the intestinal barrier. In spite of the promise shown by these observations, further research is required to elucidate if IgA ANCA may serve as a possible marker of BT.

CONCLUSION

BT plays an important role in the pathogenesis of end stage liver disease complications. Therefore, its early and reliable detection would be significant for accurately identifying a particular subset of patients with potentially adverse prognosis, who may benefit from increased vigilance and aggressive management. Several biological molecules (bacterial DNA, soluble CD14, LPS/endotoxin, lipopolysaccharide-binding protein, calprotectin, pro-calcitonin) have been tested as potential biomarkers for BT, each with its own merits and flaws (Table 1). The literature available on the subject is intriguing and expanding. In all, while no single marker has emerged as optimal for the identification of BT, there is an obvious need for better designed and more focused research. These studies will not only enable us to understand the underlying mechanisms of BT, but may also allow for implementing a timely intervention in patients with liver cirrhosis. This may, in turn, alter the natural history of this ominous disease and improve its currently unfavorable prognosis.

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REVIEW

Hepatocellular carcinoma: From clinical practice to evidence-based treatment protocols

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Abstract

Hepatocellular carcinoma (HCC) is one of the major malignant diseases in many healthcare systems. The growing number of new cases diagnosed each year is nearly equal to the number of deaths from this cancer. Worldwide, HCC is a leading cause of cancer-related deaths, as it is the fifth most common cancer and the third most important cause of cancer related death in men. Among various risk factors the two are prevailing: viral hepatitis, namely chronic hepatitis C virus is a well-established risk factor contributing to the rising incidence of HCC. The epidemic of obesity and the metabolic syndrome, not only in the United States but also in Asia, tend to become the leading cause of the long-term rise in the HCC incidence. Today, the diagnosis of HCC is established within the national surveillance programs in developed countries while the diagnosis of symptomatic, advanced stage disease still remains the characteristic of underdeveloped countries. Although many different staging systems have been developed and evaluated the Barcelona-Clinic Liver Cancer staging system has emerged as the most useful to guide HCC treatment. Treatment allocation should be decided by a multidisciplinary board involving hepatologists, pathologists, radiologists, liver surgeons and oncologists guided by personalized -based medicine. This approach is important not only to balance between different oncologic treatments strategies but also due to the complexity of the disease (chronic liver disease and the cancer) and due to the large number of potentially efficient therapies. Careful patient selection and a tailored treatment modality for every patient, either potentially curative (surgical treatment and tumor ablation) or palliative (transarterial therapy, radioembolization and medical treatment, *i.e.*, sorafenib) is mandatory to achieve the best treatment outcome.

Key words: Hepatocellular carcinoma; Evidence-based; Management; Clinical practice; Treatment allocation

Core tip: In response to the hepatocellular carcinoma (HCC) burden marked differences between countries are reflected in providing disparate quality of healthcare considering screening and surveillance programs; available treatment modalities and drugs; reimbursement of specific treatment options by the state-funded health insurance. Since the number of new HCC cases being diagnosed each year is nearly equal to the number of deaths from this cancer it is clear that the international scientific community and healthcare systems worldwide have no efficient answer to this problem. International consensus on the use of any given staging model is lacking. High-quality trials with better patients' stratification are mandatory. This review article reflects the perspective of liver surgeons working in a developing country.

Galun D, Basaric D, Zuvela M, Bulajic P, Bogdanovic A, Bidzic N, Milicevic M. Hepatocellular carcinoma: From clinical practice to evidence-based treatment protocols. *World J Hepatol* 2015; 7(20): 2274-2291 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i20/2274.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i20.2274

INTRODUCTION

According to Bailar *et al*[1] cancer mortality rates have not been significantly reduced in industrialized countries except for testicular cancer, leukemia and lymphoma in spite of an evident progress in developing innovative approaches for cancer treatment. Hepatocellular carcinoma (HCC) is a frustrating example for general disappointment with the results of cancer treatment having in mind that the growing number of new cases being diagnosed each year is nearly equal to the number of deaths from this disease[2-4].

Hepatocellular cancer is characterized by high and increasing incidence, late diagnosis when curative intent treatments are not feasible, low resectability rate, high recurrence after a curative intent surgery, poor response to medical treatments, and finally grave prognosis. These characteristics define HCC as one of the major malignant diseases in many healthcare systems worldwide. Today, HCC is one of the leading causes of cancer-related deaths, as it is the fifth most common cancer and the third most important cause of cancer related deaths in men[2-4].

A growing incidence of HCC was found in North America increasing annually by 5.4% between 2002 and 2006 being one of only four malignancies demonstrating a growing number of new cases[3,5]. Hispanics and blacks are found to have the greatest increase in incidence in the United States[6]. The overall 5-year survival less than 12% and 3-fold increase in incidence of HCC from 1975-2007 in both sexes made HCC the fastest rising cause of cancer related death in United States[7].

More than 748000 new cases are diagnosed each year, accounting for 9.2% of all new cancer cases worldwide (7.9% in men; 3.7% in women)[8-10]. Moreover, the number of new cases of HCC increases continuously[11].

Furthermore, HCC is a major burden for healthcare systems in underdeveloped countries with 84% of the world HCC population having the highest annual fatality ratio of any human tumor (0.96)[8,9,11]. Underdeveloped regions may even have a 100-fold greater incidence of HCC compared to developed countries. This is one of the greatest differences recorded among cancers[10]. Sub-Saharan Africa and Eastern Asia are regions with the greatest incidence of HCC demonstrating incidence rates of over 20 per 100000 individuals[7]. This figure is most probably even larger when considering that many HCC cases remain under-diagnosed or under-reported[11]. In these regions the most common cause for HCC is HBV transmission at birth and the diagnosis is established about one decade earlier compared to the developed countries characterized by HCV acquired later in life as a dominant cause for HCC[7].

Mediterranean countries have intermediate incidence rates of 10-20 per 100000 individuals, while North and South America have a relatively low incidence despite of the reported increase in the number of HCC cases (< 5 per 100000 individuals)[7].

In developed countries, HCC dominantly occurs in patients over 60 years old while in underdeveloped regions the HCC diagnosis is already established in many patients in their 30 s[9-11]. In all regions, there is a predominance of the male over the female gender (3/4:1) in the Asia-Pacific region, sub-Saharan Africa and medium-risk countries, compared to 2:1 in regions with a low incidence of HCC[8-11].

The majority of HCC cases occur in cirrhotic livers[10,12,13]; therefore the competing mortality risks from the tumor and the cirrhosis should be considered when deciding for a specific treatment modality.

In the majority of countries worldwide the diagnosis is established late when only limited treatment options are available resulting in poor treatment outcome. Only in Japan the strict adherence to the national surveillance program led to improved treatment results. This is mainly because approximately 20% of HCC cases are diagnosed in an early stage when curative treatment modalities can be applied[14,15].

In response to the HCC burden marked differences between countries worldwide are reflected in providing disparate quality of healthcare considering screening and surveillance programs; available treatment modalities and drugs; reimbursement of specific treatment options

by the state-funded health insurance.

RISK FACTORS AND ETIO-PATHOGENESIS

Viral hepatitis, namely chronic hepatitis C virus (HCV) is a well established risk factor contributing to the rising incidence of HCC[16]. The epidemic of obesity and the metabolic syndrome, not only in the United Stated[17] but also in Asia[18], tend to become the leading cause of the long-term rise in HCC incidence.

HCV is an important global risk factor for HCC, especially in developed countries, compiling more than 170 million of people being chronically infected world-wide[19,20]. The dominant prevalence is among injecting drug users (60%-90%); hemophiliacs (50%-70%); hemodialysis patients (15%-60%); and patients who received blood transfusions before 1991 (5%-10%)[21]. About 25% of patients having chronic HCV infection will develop cirrhosis and significant proportion will progress to HCC with a time interval of about 20 years or longer[19-21].

HCV-related carcinogenesis is mediated by inducing hepatic inflammation and later fibrosis; and finally by promoting malignant transformation of infected cells[22].

Approximately 55% of all worldwide HCC cases are associated with chronic hepatitis B virus (HBV) infection[8]. Among 400 million people chronically infected with HBV, about 25% will develop HCC[2,8]. Chronic HBV infection distribution is nearly parallel to HCC high-risk regions and it is implicated in the development of 85% of HCC cases among ethnic Chinese and the Black African population[2,23]. While in the developed countries, HCC is rare before the age of 40 irrespective of the HBV status, in underdeveloped countries, there is a distinct shift toward a younger age[2,23,24]. A study from China on Han Chinese population characterized by high prevalence of HBV infection demonstrated that polymorphism of *GRP78* gene (genotypes AA and AG of rs430397) is associated with the development and prognosis of HCC[25].

HBV-induced carcinogenesis is essentially an inflammatory process resulting from the reaction of the host's immune response to the presence of the virus. Integration of HBV DNA into host DNA is considered a critical step in HBV related HCC[26,27]. This leads to series of changes like cell cycle progression, inactivation of negative growth regulators, inhibition of the expression of p53 tumor suppressor gene and other tumor suppressor genes[26,27].

Recently, a striking increase in the incidence of obesity was recorded parallel to the increase in the incidence of HCC in several developed countries[28,29]. The increase in the number of HCC related cancer deaths in the United States has been documented while at the same time it is estimated that 25% of the population meet the diagnostic criteria for the metabolic syndrome[30]. In the great majority of the obese patients, the obesity is attributed to the metabolic syndrome. A recent meta-analysis demonstrated that the relative risk for HCC is 1.17 (95%CI: 1.02-1.34) in those who were overweight [body mass index (BMI) 25-30 kg/m^2] and 1.89 (95%CI: 1.51-2.36) in those who were obese (BMI > 30 kg/m^2)[31]. The incidence of the metabolic syndrome continues to increase in developed countries whereas the highest incidence is believed to occur in the United Kingdom (34% of the adult population)[32]. While obesity is present in up to 100% of patients with non-alcoholic fatty liver disease (NAFLD), the risk of liver steatosis is much higher in obese than in non-obese patients[5,30]. Finally, patients with liver steatosis are at high risk for developing cirrhosis and HCC[33]. Although NAFLD is currently the most common liver disease in developed countries, the incidence of HCC associated with NAFLD is lower than HCC associated with non-alcoholic steatohepatitis (NASH) (4%-27%)[33,34]. Today, the risk of HCC developing in NASH-cirrhotic patients challenges the risk of HCC developing in HCV-cirrhotic patients[35].

The pathogenesis linking obesity, NAFLD, NASH and HCC is still a subject of research. The relationship between obesity and HCC are thought to be mediated by factors associated to metabolic syndrome, NAFLD and NASH[17]. There is growing evidence that links obesity to chronic liver inflammation[17]. Moreover it is found that an excessive accumulation of fatty acids and glucose lead to increased expression of tumor necrosis factor-α, nuclear factor-kappa B, EGF heading to hepatic inflammation[36,37].

One other finding is that adipose tissue induces expression of leptin, a hormone that regulates body mass[38]. In animal models it was shown that leptin promotes angiogenesis and mediate the progression of NASH to HCC[38]. Leptin is found to upregulate JAK/STAT, AKT and ERK, *i.e.,* signal transduction pathways involved in cancer progression in HCC cells[39].

Moreover, leptin levels are increased in patients with NASH, what may explain an increased vascular invasiveness in HCC patients with metabolic syndrome[40].

Aflatoxins is another risk factor for HCC. These toxins are metabolites of the widely distributed fungi *Aspergillus flavus* and *Aspergillus parasiticus* and their toxic, teratogenic, mutagenic and carcinogenic properties pose a serious risk to humans[41-43]. Approximately 4.5 to 5.5 billion people worldwide are at risk of exposure dominantly in Sub-Saharan Africa, Eastern Asia, and parts of South America[41,43]. Contamination occurs either in tropical and subtropical climates or in conditions where food drying and storage facilities are suboptimal. Aflatoxins are responsible for between 4.6% and 28.2% of all HCC cases worldwide[43]. The AFB1 toxin is metabolized in the liver by p450 enzymes forming AFB1-8,9-exo-epoxide, which further react with the p53 tumor suppressor gene[44,45]. Mutation at codon 249 of the p53 tumor suppressor gene accounts for 90% of p53 mutations in AFB1-related HCC[46]. There is a direct correlation between the degree of exposure to AFB1 and the incidence of HCC[42].

The study from Yu *et al*[47] found a synergistic effect of AFB1 and HBV in causing HCC since population with HBV who lived in the region of high exposure to AFB1 were associated to a mortality rate ten times higher than that of population with HBV living in the region of low exposure to the toxin.

Alcohol abuse, lasting more than 10 years, increases the chance for HCC development approximately five fold[48]. It is most common in the Americas (32% of HCC cases in the United States)[48] and Western Europe (45% of the cases in Italy[49]) and the incidence is increasing in Asia[7,9]. In principle, patients who develop the tumor have alcohol-induced cirrhosis[50].

Other less frequent risk factors include iron overload[51], hereditary hemochromatosis[52], tobacco smoking[53,54] and membranous obstruction of inferior vena cava[55].

DIAGNOSIS

Today, the diagnosis of HCC is established within the national surveillance programs in developed countries while the diagnosis of symptomatic, advanced stage, disease still remains the characteristic of underdeveloped countries. According to the American Association for the Study of Liver Diseases (AASLD) screening for HCC is recommended according to existing guidelines in all cirrhotic patients using ultrasound every six months[56]. Screening for chronic HBV carriers is recommended as well[57].

When a nodule is detected in a cirrhotic liver, a contrast-enhanced diagnostic procedure is strongly recommended. It is important to search for the typical signs of HCC (arterial phase enhancement and portal venous phase washout)[56]. The updated guidelines of AASLD consider that a non-invasive diagnosis of HCC can be established if a lesion > 10 mm has a typical vascular enhancement pattern in 4-phase multi-detector row CT (MDCT) or dynamic contrast enhanced magnetic resonance imaging (DCE-MRI)[56]. These guidelines were also accepted by the European societies[58].

Although MDCT is currently the most common imaging modality for detecting HCC, it is suboptimal for nodule characterization. DCE-MRI, with liver-specific contrast agents, has emerged as the preferred diagnostic modality for the investigation of HCC as it facilitates liver cancer characterization[59-61]. A recent meta-analysis[62] estimated the accuracy of MRI with liver-specific contrast agents compared to MDCT for the detection and characterization of HCC and demonstrated the superiority of MRI for the detection of HCC lesions < 20 mm.

For nodules smaller than 1 cm, a repeated ultrasound examination in three months intervals is recommended[56]. A biopsy is required only if imaging is inconclusive for lesions smaller than 2 cm, or it is atypical for lesions larger than 2 cm when the AFP level is not elevated[56]. However, biopsy carries an approximately 2% risk of tumor seeding[63] and the false-negative rate can be greater than 10% for small lesions[64]. The AASLD guideline has been prospectively validated for focal lesions 0.5 to 2.0 cm in size using MRI and contrast-enhanced ultrasound, and demonstrated a low sensitivity (33%) but a very high specificity (100%) for the diagnosis of HCC[65].

STAGING

Since 1984 nine different staging systems have been developed and evaluated. The Barcelona-Clinic Liver Cancer (BCLC) staging system has emerged as the most useful to guide treatment decisions (Figure 1). BCLC is based on the analysis of independent studies in different clinical settings. It includes prognostic variables related to tumor status, liver functional status, and health performance status, together with treatment-dependent variables obtained from cohort studies and randomized clinical trials. The system links tumor stage with the treatment strategy allowing an estimation of life expectancy associated to specific HCC management[66].

BCLC demonstrated the best independent predictive power in many trials[67-71] when the entire patient population was included [not limited to patient population treated by surgery, radiofrequency ablation (RFA) or transarterial chemoembolization (TACE) only]. The BCLC staging system was externally validated[67,68,71,72] and has practically become an universal staging and treatment system. Moreover it was endorsed by European Association for the study of the liver (EASL) and AASLD as standard for patients with HCC[56,66].

However, other trials have demonstrated conflicting results thus favoring other staging systems[73-77]. Graf *et al*[78] have shown many limitations for the BCLC staging system (Table 1). Furthermore, as indicated by Maida *et al*[79] the BCLC staging system was not derived from a cohort of HCC patients by a multivariate analysis, and therefore it is not a prognostic model able to predict the mortality of HCC patients. Moreover, the intermediate stage (BCLC B) includes an extremely heterogeneous population in terms of both liver function and tumor characteristics and the main limitation of the BCLC is represented by its rigidity when it is acting as a treatment algorithm[79].

Importantly, treatment allocation should be decided by a multidisciplinary board based on individualized rather than on a guideline-based approach[80].

Although BCLC is the most comprehensive staging system, as it integrates tumor status, liver function and the performance status neither BCLC nor any other of the staging systems has been universally accepted, as pointed out by the AASLD guidelines[56], meaning that international consensus on the use of any given model is lacking.

TREATMENT

Treatment allocation should be decided by a multidisciplinary board involving hepatologists, pathologists,

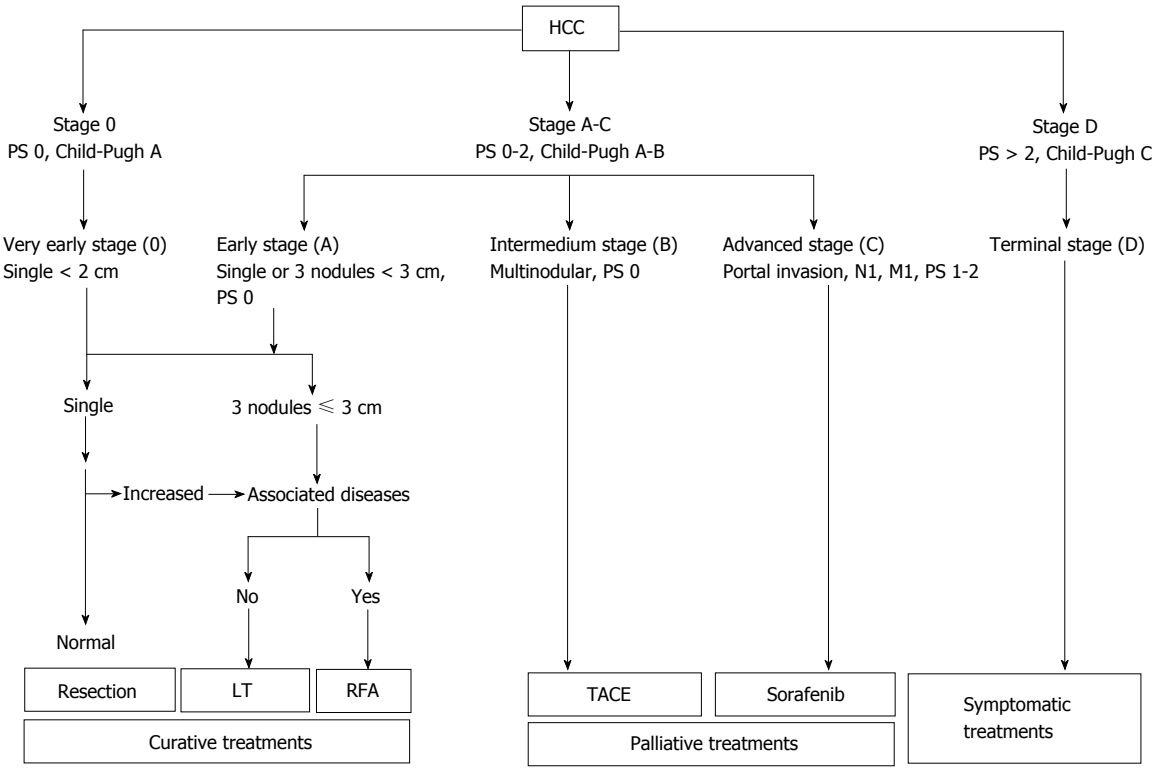

Figure 1 Barcelona-clinic liver cancer staging system. BCLC: Barcelona-clinic liver cancer; TACE: Transarterial chemoembolization; HCC: Hepatocellular carcinoma; LT: Liver transplantation; RFA: Radiofrequency ablation.

Table 1 Limitations of the barcelona-clinic liver cancer staging system[78]

No	BCLC classification system
1	Does not consider nodule location, which is essential for defining respectability
2	Does not respect etiology of cirrhosis
3	Is based on variables measured at diagnosis, which might change over time
4	Does not consider the possibility of liver transplantation for patients with Child C cirrhosis with hccs within the Milan criteria
5	Does not reflect contraindications of TACE
6	Recommends liver resection to single nodules only in absence of portal hypertension in very early (BCLC 0) and early stage (BCLC A), however probably portal hypertension might not affect survival in resected patients
7	Recommends liver resection in very early (BCLC 0) and early stage (BCLC A), however in selected patients hepatic resection is associated with good survival even in more advanced BCLC stages
8	Does not consider treatment sequences or combination therapies
9	Includes a very heterogeneous population in the intermediate stage (BCLC B) in respect to tumor burden and liver function
10	Does not consider other therapies than sorafenib in selected patients with advanced stage C with performance status 1
11	Is not favorable as classification system in non-cirrhotic patients

BCLC: Barcelona-clinic liver cancer; TACE: Transarterial chemoembolization.

radiologists, liver surgeons and oncologists guided by personalized-based medicine by. This approach is important not only to balance between different oncologic treatments strategies but also due to the complexity of the disease (combination chronic liver disease and the cancer) and due to the large number of potentially efficient therapies. When considering different treatment options the following is important: (1) there is a marked difference in available treatment modalities from one country to another; (2) historic studies are lacking, *i.e.*, the results of potentially curative treatment modalities have never been compared to no treatment - today such studies are unethical; (3) the level of evidence for certain treatment modalities is limited to cohort studies and only a few randomized controlled trials; and (4) large, robust studies comparing results of different treatment modalities offered to patients in early stage disease are lacking as well.

Surgical treatment

Surgical treatment of HCC is established as a potentially curative treatment modality and includes liver transplantation, liver resection for HCC in cirrhotic livers and liver resection for HCC in non-cirrhotic livers.

Liver transplantation

Liver transplantation (LT) is the best treatment option as it removes both the tumor and the diseased liver

parenchyma[81,82]. This is primarily important for patients with a Child-Pugh (CP) score C as it is the treatment of liver failure. The patient's age (typically younger than 70 years), co-morbidities (*e.g.,* cardiopulmonary disease, smoking, diabetes or renal disease), nutritional state (*e.g.,* poor nutrition or morbidly obese), and social factors (*e.g.,* adequate support, compliance, abstinence from alcohol and completion of an appropriate rehabilitation program) are all factors determining the patients' eligibility for LT[81].

The most appropriate candidates for LT are patients that fit into the Milan criteria (a single tumor < 5 cm or up to 3 tumors of < 3 cm) achieving a 5-year survival rate of 70%-80%. In these patients the recurrence rates are approximately 10%[83,84].

The Milan criteria can be expanded to include more patients primarily by liberalizing the restrictions on tumor size. Yao *et al*[85] demonstrated that using the University of California San Francisco criteria (single nodule < 6.5 cm or ⩽ 3 nodules each ⩽ 4.5 cm, with total combined tumor diameter ⩽ 8 cm), a 75% 5-year survival rate is achievable. Kaido *et al*[86] reported that using the Kyoto criteria (a combination of tumor number ⩽ 10, maximal diameter of each tumor ⩽ 5 cm, and serum des-γ-carboxy prothrombin levels ⩽ 400 mAU/mL), the 5-year survival rate after living donor LT is 82%.

Mazzaferro *et al*[87] have proposed the "Metro ticket price" concept - the further one goes in expanding the criteria for LT, the more one "pays", *i.e.,* the more you deviate from the Milan criteria, the survival rate decreases and recurrence rate increases.

Due to the limited number of donors and the scarcity of sufficient available data, current guidelines do not recommend LT for HCC patients outside the Milan criteria[58,88]. Patients with a compromised liver function (CP - B or C) should be listed for LT while allocation of this treatment modality to CP class A patients instead of surgical resection is still an area of debate. The Barcelona Clinic has analyzed their results for surgical resection and LT in an intent-to-treat manner, although the patients were never compared directly in a randomized trial[89]. The five-year survival rates for resection and LT were nearly identical if patients for resection were carefully selected (CP class A, normal bilirubin levels and no portal hypertension).

Waiting time for LT is a serious obstacle in many national transplant programs worldwide. When the waiting list for LT is longer than 12 mo the drop-out rates can reach 25% of HCC patients listed for LT[90,91]. Clearly, if patients with more advanced tumors are included as a result of expanded listing criteria the dropout rate will be higher and this will lead to poor survival figures. In that regard the potential benefit of TACE, TARE, RFA and others, applied in the neoadjuvant setting include "bridging" or "down-staging" strategies to increase the number of HCC patients qualifying for LT[92].

Furthermore another important concept of LT is salvage LT that saves the donor pool and can effectively be performed for patients with recurrence or liver function deterioration following resection for HCC. This does not increase the perioperative mortality and has similar long-term survival compared to primary LT[93].

Liver transplantation can also be offered to patients with non-resectable HCC in normal livers providing 5-year survival rates of 59%[94]. In contrast to LT for HCC in cirrhosis the tumor size is not a predictor of post-transplant survival[94].

Finally, many controversies related to LT were confronted during an international consensus conference held in 2010, in Switzerland that resulted in 37 statements and recommendations[95]. These recommendations reflect the current state of scientific evidence regarding the LT and reflect differences in clinical practice of LT between continents, countries and institutions. In each controversial topic the strength of recommendation was conditioned by the level of evidence that was in the majority of instances 2 or less reflecting the quality of evidence that is currently available. Among the 37 recommendations only 17 are strong (presented in Table 2) while the others are week or their strength could not be established due to insufficient data[95].

The highest level of evidence and the strength of recommendation is related to the assessment of candidates for LT and in defining criteria for listing candidates with HCC in cirrhotic livers for deceased donor LT. In regard to HCC patients in non-cirrhotic liver LT this procedure may be considered as salvage transplantation for patients with intrahepatic recurrence following liver resection and no evidence of lymph node or macrovascular invasion[95].

The role of down-staging was evaluated in perspective of different loco-regional treatment options that are presented in the literature (TACE, TARE, RFA). Although the largest experience is linked to TACE and RFA, based on existing evidence, no recommendation can be made for selecting a specific loco-regional therapy for down-staging[95].

Living donor LT is an important alternative to deceased donor liver transplantation in the present circumstances of increasing number of HCC patients listed for LT. It is conducted in a limited number of centers worldwide. Although it facilitates access to LT, recent meta analysis demonstrated that living donor LT is associated with a higher rate of surgical complications following transplantation[96].

In that sense an important recommendation is derived from the consensus conference, *i.e.,* that living donor LT must be restricted to centers of excellence in liver surgery and liver transplantation to minimize donor risk and maximize recipient outcome[95].

Liver resection

During the past decade a tremendous improvement in the understanding of liver anatomy, advances in technology, anesthesiology and postoperative intensive care and the application of intraoperative ultrasono-

Table 2 Recommendations from international consensus conference on liver transplantation (only the recommendations with the highest level of evidence are presented, adopted from Clavien *et al*[95])

Assessment of candidates with HCC for liver transplantation
When considering treatment options for patients with HCC, the BCLC staging system is the preferred staging system to assess the prognosis of patients with HCC
The TNM system (7th ed) including pathological examination of the explanted liver, should be used for determining prognosis after transplantation with the addition of assessment of microvascular invasion
Either dynamic CT or dynamic MRI with the presence of arterial enhancement followed by washout on portal venous or delayed imaging is the best non-invasive test to make a diagnosis in cirrhotic patients suspected of having HCC and for preoperative staging
Extrahepatic staging should include CT of the chest, and CT or MRI of the abdomen and pelvis
For patients with lesions smaller or equal to 10 mm, non-invasive imaging does not allow an accurate diagnosis and should not be used to make a decision for or against transplantation
Criteria for listing candidates with HCC in cirrhotic livers for deceased donor LT
Preoperative assessment of the size of the largest tumor or total diameter of tumors should be the main consideration in selecting patients with HCC for liver transplantation
The Milan criteria are currently the benchmark for the selection of HCC patients for liver transplantation, and the basis for comparison with other suggested criteria
Biomarkers other than α-fetoprotein cannot yet be used for clinical decision making regarding liver transplantation for HCC
Indication for liver transplantation in HCC should not rely on microvascular invasion because it cannot be reliably detected prior to transplantation
Role of down-staging
Liver transplantation after successful down-staging should achieve a 5-yr survival comparable to that of HCC patients who meet the criteria for liver transplantation without requiring down-staging
Criteria for successful down-staging should include tumour size and number of viable tumours
Managing patients of the waiting list
Periodic waiting-list monitoring should be performed by imaging (dynamic CT, dynamic MRI, or contrast-enhanced US) and α-fetoprotein measurements
Patients found to have progressed beyond criteria acceptable for listing for liver transplantation should be placed on hold and considered for down-staging
Patients with progressive disease in whom locoregional intervention is not considered appropriate, or is ineffective, should be removed from the waiting list
Role of living donor LT
Living donor LT must be restricted to centers of excellence in liver surgery and liver transplantation to minimize donor risk and maximize recipient outcome
In patients following living donor LT for HCC outside the accepted regional criteria for deceased donor LT, re-transplantation for graft failure using a deceased donor organ is not recommended
Post-transplant management
Liver re-transplantation is not appropriate treatment for recurrent HCC

BCLC: Barcelona-clinic liver cancer; TACE: Transarterial chemoembolization; HCC: Hepatocellular carcinoma; LT: Liver transplantation; CT: Computed tomography; MRI: Magnetic resonance imaging.

graphy have established surgical resection as a widely accepted first-line curative treatment option for HCC patients. Surgical resection for HCC is a safe and reliable procedure and, unlike LT, it is available in many countries and institutions. Presently, when considering liver resection, the main focus has shifted from the tumor towards the functional capacity of the remnant liver.

Liver resection for HCC is considered in two different settings. One is liver resection for HCC in non-cirrhotic, "normal" livers and the other is liver resection in cirrhotic livers, when special attention is attributed to the functional capacity of the remnant liver. Considering the improvement in the technical feasibility of complex liver resection there are practically no more non-resectable tumors, but considering the functional capacity of the remnant liver only a relatively small percentage of HCC patients with cirrhotic livers can be offered curative-intent liver resection.

Patients with HCC in non-cirrhotic livers are rare in the western world; only 5%-15% of HCC patients have a normal, non-cirrhotic parenchyma[58,97,98]. They are diagnosed late with large-size tumors sometimes with major vascular invasion. Liver resection is the only curative treatment in these patients and up to 70%-80% of functional liver parenchyma can be removed[78].

The 5-year disease free survival of non-cirrhotic HCC patients managed by liver resection is around 50% depending on resection status, UICC stage, vascular invasion, tumor size > 10 cm and tumor grading[98-101]. About 50% of these patients will have recurrence within 2 years after curative resection[58,101]. Repeated liver resection is the treatment of choice for patients with intrahepatic recurrence having a similar prognostic outcome as the primary resection[102].

According to the BCLC staging system, surgical resection for HCC in cirrhosis is reserved for patients in the BCLC 0 stage (single tumor < 2 cm, Child A, ECOG 0 without portal hypertension and normal bilirubin level) and it is feasible in selected patients in the BCLC A stage. However, clinical practice worldwide (not only in Japan) is not limited to the frame recommended by the BCLC staging. Moreover, it is expanded even to selected patients belonging to BCLC intermediate stage B group. This has to be considered within a context that in many developing countries screening and surveillance programs are lacking, therefore the majority of patients

are diagnosed in an advanced stage of the disease when surgical resection is still feasible[103,104]. Strict adherence to the BCLC staging system would direct the majority of patients to palliative treatment only.

Despite of recent advances in surgical techniques and perioperative care, liver resection is challenged by the poor functional reserve of the cirrhotic liver, the impaired regeneration capacity, elevated portal venous pressure, and other co-morbidities of the HCC patients[105,106]. Although reserved for high-volume centers, liver resection is justified even for patiens with large and multinodular HCC[107-109].

A study from Ishizawa *et al*[110] has demonstrated that neither multiple tumors nor portal hypertension are surgical contraindications for HCC. Two other studies have verified that liver resection is feasible even in Child B patients and in selected patients a major liver resection is feasible as well[111,112]. According to Ho *et al*[113] liver resection is associated with better overall survival comparing to TACE (37.9 mo *vs* 17.3 mo) even for patients with multinodular HCC. In patients with large tumors, TACE is associated with low response rate and a modest 3 years survival rate[108,109].

Several studies confirmed that blood loss has a negative impact on the perioperative morbidity, mortality and long-term outcome[114,115] therefore a control of bleeding is mandatory when performing liver resection. Vascular occlusion techniques[116] are effective in reducing blood loss, but it was found that they compromise hepatic functional reserve in conditions of a preexistent liver disease[117,118]. Fu *et al*[119] found an earlier recovery of the postoperative liver function after hemihepatic vascular inflow occlusion compared with the Pringle maneuver, however it is technically more demanding and potentially associated with more bleeding in cirrhotic livers.

Prediction of the future, functional remnant liver volume (FLR) is crucial for postoperative morbidity and mortality. A remnant volume of at least 40% should remain following resection of cirrhotic livers in order to preserve adequate liver function[120]. Three dimensional measurements of liver volumes based on MDCT or magnetic resonance imaging (MRI) and more important post-processing software are important for predicting the FLR after liver resection.

Portal vein embolization (PVE) has an important role as an effective tool in inducing hypertrophy of the non-embolized hepatic segments. An increase of the FLR volume of 20%-46% can be achieved after 2-8 wk[121,122]. When the FLR volume is insufficient PVE is considered an important therapeutic step before extended resection. Recently, one other approach has been described for increasing the FLR volume in a two-stage procedure for patients undergoing extended liver resection. *In situ* liver transection combined with portal vein ligation emerged as a procedure associated with rapid growth of the FLR[123,124] and was tested in the settings of HCC in cirrhotic livers[125] even in conditions of major vascular invasion[126]. The median FLR volume increase was 18.7% within one week after the first step and 38.6% after the second step[125]. More studies are needed before the real merits of ALPPS can be evaluated.

The use of metabolic tests, namely the indocyanine green test is another tool to assess the liver functional capacity in order to avoid postoperative liver failure[127]. As indicated in two surveys[120,128] it is widely used in Asia and the indocyanine green retention rate at 15 min (ICGR-15) is integrated into the decision tree for deciding the safe limit of hepatectomy[127]. In the western world the ICGR-15 test is used in a limited number of centers and in selective cases only[120]. In HCC patients with cirrhotic livers characterized by normal bilirubin level and absence of ascites the ICGR-15 is the main determinant for performing a liver resection[127].

The anatomic liver resection should be associated with improved outcome as HCC tumors have a tendency for local portal vein invasion with possible extension toward the main portal vein. However conflicting results are present in the literature. Two studies[129,130], demonstrated that anatomic resection is an independent predictor of improved recurrence-free survival and it significantly improves the disease-free survival rates. Anatomic resection is recommended in the EASL guidelines as the preferred approach if sufficient remnant liver volume can be preserved[56]. The use of dye widely practiced in Japan may aid delineation of tumor bearing segments and facilitate complete anatomical resection[131,132].

Laparoscopic liver resection for HCC in cirrhotic livers is an established and safe procedure performed in many centers worldwide[128]. There are no randomized controlled trials that has compared laparoscopic *vs* open liver resection in HCC patients. Four meta-analyses[133-136] of nonrandomized studies found that laparoscopic resection was associated with significantly less blood loss, lower transfusion requirements, lower overall morbidity, and shorter length of hospital stay without a significant difference in length of operation, surgical margin status, or tumor recurrence rates.

Ablative procedures

Tumor ablation can be achieved by chemical (ethanol, acetic acid) or thermal [radiofrequency ablation-RFA, microwave ablation (MWA)] ablation and it is the treatment of choice in patients with single, small tumors who are not candidates for surgery. According to the BCLC staging and treatment algorithm these patients are classified as BCLC A patients[58]. BCLC 0 patients may also be managed by this treatment modality, although the algorithm primarily allocates resection to this group of patients[58]. When procedure limitations are strictly respected (tumor size, tumor location, duration of the treatment, maintaining the required temperature in the tumor zone, *etc.*) tumor ablation is a curative treatment option for the management of carefully selected HCC patients.

Historically, tumor ablation started as chemical

ablation using percutaneous ethanol injection (PEI) for the management of nodular-type HCC. There is considerable experience with PEI since it is an established technique. PEI induces coagulation necrosis of the tumor as a result of cellular dehydration, protein denaturation, and chemical occlusion of small tumor vessels[137]. Several studies confirm that tumors < 2 cm can be successfully treated by PEI achieving equivalent results to thermal ablation techniques[137-139]. For larger tumors PEI is inferior to thermal ablation and therefore should not be performed[138-142]. However, PEI should not be neglected and can be used in underdeveloped regions as a very useful treatment modality.

Thermal ablation has now largely replaced PEI, initially with RFA and recently with MWA[137,139]. Although it is an interventional procedure performed percutaneously by interventional radiologists or jointly by an interventional radiologist and liver surgeon, a multidisciplinary approach which provides important advantages, as described by Poon *et al*[143]. Thermal ablation can also be done *via* an open or laparoscopic surgical approach.

The main advantage of thermal ablation is related to its low major morbidity (2.2%-3.1%) and mortality (0.1%-0.5%) rates[144,145]. Major complications include intraperitoneal hemorrhage, hepatic abscess, bile duct injury, and liver decompensation[56,144,145]. Tumor seeding along the needle track has been reported as a rare (0.5%) late complication of RFA[146].

The most important observations resulted from explants studies following LT and demonstrated complete tumor necrosis in explanted liver specimens in 83% of tumors > 3 cm and in 88% of tumors in non-perivascular locations[54,56,144,145]. Clearly the efficacy of RFA is reduced with increasing tumor size and the presence of large vessels[147]. RFA should be applied for tumors less than 3 cm in size, bearing in mind that success is related to the total volume of the tumor tissue that has to be ablated.

Lencioni *et al*[145,148] have demonstrated 61% 5-year survival in patients with Child A cirrhosis and solitary HCC, compared with 51% in patients with Child A cirrhosis and multiple tumors and 31% in patients with Child B cirrhosis. Livraghi *et al*[149] has reported complete tumor response in 97% of tumors ⩽ 2 cm, with 5-year survival in patients with preserved hepatic function of 68%, challenging resection as the first-line approach in such cases.

Hasegawa *et al*[150] concluded that resection was associated with a higher overall survival and lower recurrence rate than RFA or PEI in the treatment of HCC ⩽ 3 cm.

A challenging question is whether emerging alternative, MWA, will replace RFA. Compared to RFA, MWA is less-susceptible to the heat sink effect of nearby blood vessels and produces a larger zone of necrosis[151].

In a non-randomized study published in 2013[152] that investigated the therapeutic efficacy of percutaneous RFA and MWA for HCC < 5 cm no significant differences were found between the two procedures in the percentage of complete ablation local tumor progression, distant recurrence and overall survival. Clearly, more studies are needed to compare the two ablation techniques.

Transarterial therapy

According to the BCLC staging and treatment algorithm TACE is indicated for patients classified as BCLC B stage, that is an intermediate stage composed of a very heterogeneous patient population[56,153,154]. A Cochrane review[154] clearly confirmed the survival benefit of this treatment modality. However, TACE is not standardized in regard to: (1) the procedure technique; (2) the choice of embolic agent; (3) the choice of applied medications; and (4) the schedule (on demand or at fixed intervals). In clinical practice TACE is performed by injection of chemotherapy with or without lipiodol, followed by the injection of embolic particles. This procedure is considered as conventional TACE. Innovative step forward was the development of drug-eluting beads (DC Bead) used to increase tumor drug delivery. However, the PRECISION V study[155] designed to compare the two TACE procedures failed to demonstrate a clear superiority of DC Bead-TACE (one-sided $P = 0.11$). The difference between the two TACE procedures was found in the complete response, objective response, and disease control favoring DC Bead group (27% *vs* 22%, 52% *vs* 44%, and 63% *vs* 52%, respectively)[155].

Complications of TACE include non-target embolization, the post embolization syndrome (fever, abdominal pain, ileus), liver failure, cholecystitis and acute portal vein thrombosis[154]. The procedure-related mortality is less than 5% which defines TACE as a safe procedure[154]. Main portal vein thrombosis, poor liver function, and extrahepatic spread have been shown to be predictors of poor outcome and are considered contraindications for chemoembolization[154].

Several aspects of TACE treatment require special consideration. In clinical practice an attempt should be made to achieve the supraselective approach (STACE) using micro-catheters in order to deliver chemotherapy as close as possible to the tumor site. Unfortunately this aspect was not much elaborated in clinical trials. Only one trial[156] on 60 patients who were candidates for LT, found STACE to be associated with complete tumor necrosis in a larger proportion of patients (30.8% *vs* 6.9%, $P = 0.02$) compared to selective TACE group. Still, a 5-year disease-free survival was similar in both groups (76.8% *vs* 74.8%)[156]. In conclusion, there is no clear relationship between the therapy-induced complete necrosis and long-term survival.

The combination of TACE and RFA is another challenging treatment option practiced in many centers worldwide. Recent meta-analyses[157] showed that the combination of RFA and TACE was associated with a significantly higher overall survival rates (OR 1 year = 2.39, 95%CI: 1.35-4.21, $P = 0.003$; OR 3 years = 1.85, 95%CI: 1.26-2.71, $P = 0.002$), and recurrence-free survival rate (OR 1 year = 2.00, 95%CI: 1.26-3.18, *P*

= 0.003; OR 3 years = 2.13, 95%CI: 1.41-3.20, P < 0.001) compared with RFA alone[157]. The quality of the evidence was high for the 1- and 3-year survival[157].

Two recent meta-analysis[158,159] elaborated the combination of TACE and sorafenib and found evident improvement in the objective response, time to progression and overall survival although the sorafenib associated AEs were more frequent in the combination therapy group[158,159].

Another important meta-analysis[160] examined the efficacy of TACE for HCC patients with portal vein thrombosis (PVTT) and found that TACE improves the 1-year survival of patients with HCC and PVTT. As current treatment algorithms contraindicate TACE in patients with main trunk PVTT more trials are required to confirm these findings[54,56,66].

As already indicated the BCLC B stage (an intermediate stage) is composed of a very heterogeneous patient population. Several studies confirmed limited treatment efficacy of TACE for HCC patients with large multinodular tumors[161,162]. The objective response to TACE treatment is found in 52% of patients (PRECISION V trial[155]) leaving large proportion of patients without an effective treatment. It is important to note that TACE is contraindicated in patients with main trunk PVTT. These findings led to development of new therapies for optimal management of these subcategories of HCC patients belonging to BCLC B stage[163].

Radio-embolization *via* hepatic artery using microspheres impregnated with yttrium-90 (TARE) is a new emerging treatment option that is available in a limited number of centers worldwide[162,163]. TARE uses the same concept as TACE in regards the technical aspect of the procedure. The difference is reflected in the mode of action. In TARE the embolic particles (microspheres) are 3-10 times smaller than those used in TACE (25-35 micron in diameter)[164]. Yttrium-90 (beta emitter with a short half-life) microspheres are used to produce tumor necrosis by internal delivery of tumoricidal dose of radiation directly to the tumor with nearly no embolic effect on the vessels[165]. The safety and efficacy of TARE is well established in many trials[164,166-168] and post-embolization syndrome is found in 20%-55% of cases[164,166]. TARE was found to be a safe procedure in HCC patients with PVTT[169,170]. Initially, TARE was indicated in HCC patients who progressed or relapsed after the TACE treatment or in HCC patients not amenable to TACE (large multinodular tumors or presence of PVTT)[164]. Although Sangro *et al*[162] found survival benefit for TARE comparing to TACE as a first-line treatment other studies reported no significant difference in survival[168,171,172]. Potential advantage of TARE over TACE can be attributed to early-stage HCC patients listed for LT who are candidates for bridging or down-staging therapy[173,174].

MEDICAL TREATMENT

After years of disappointment with the results of numerous trials testing the efficacy of different drugs in the medical management of HCC, two milestone studies[175,176] have established sorafenib as a treatment of choice for BCLC C patients according to the EASL-EORTC guidelines[56].

Sorafenib is a molecular inhibitor of several tyrosine protein kinases (VEGFR and PDGFR); Raf kinases (C-Raf than B-Raf)[177,178] and intracellular serine/threonine kinases (C-Raf, wild-type B-Raf and mutant B-Raf)[179] Sorafenib treatment induces autophagy[180], which suppresses tumor growth.

Although sorafenib was introduced as well-tolerated drug a subanalysis of the two leading[175,176] and other[181,182] studies have shown that the tolerability of sorafenib was suboptimal[183]; it was down-dosed in more than 50% and interrupted in 45% of patients due to severe adverse events or compromised liver function[183].

Therefore the most important side effects are gastrointestinal[184] (diarrhea 43%, increased lipase 41%, increased amylase 30%, nausea 23%, anorexia 16%, vomiting 16%, and constipation 15%), dermatologic[185] (rash/desquamation 40%, hand-foot skin reaction 30%, alopecia 27%, pruritus 19%, and dry skin 11%), cardiovascular[186]. (Hypertension 17%, angioedema, and congestive heart failure), hematologic[187]. (Hypoalbuminemia 49%, hemorrhage 15%, anemia and thrombocytopenia) and nervous system side effects[186] (neuropathy 13% and headache 10%).

One of the two milestone studies (SHARP/phase Ⅲ)[175] conducted in the western world have shown that sorafenib prolonged median survival from 7.9 mo (placebo group) to 10.7 mo (sorafenib group) (HR = 0.69; 95%CI: 0.55-0.87; P = 0.00058). Sorafenib also improved the time to progression (from 2.8 mo to 5.5 mo). Another milestone study conducted in Asia confirmed the outcomes of the SHARP trial, *i.e.,* a phase Ⅲ Asia-Pacific trial[176] have shown a median overall survival of 6.5 mo (treatment group) comparing to 4.2 mo (placebo group) (HR = 0.68; 95%CI: 0.50-0.93; P = 0.014).

Another important study was a phase Ⅳ, GIDEON trial[188], conducted with the aim to evaluate the safety of sorafenib treatment in HCC patients in real-life conditions. In 2011 the second interim analysis showed a median survival of 10.3 mo for Child A patients and 4.8 mo for Child B patients. The amount of adverse events was comparable to the two milestone studies.

The use of sorafenib in adjuvant settings was addressed in the STORM trial. In mid 2014 major pharmaceutical companies Bayer and Onyx announced that the STORM trial did not meet its primary endpoint. During the ASCO annual meeting in 2014 Bruix *et al*[189] reported that both primary and secondary endpoints were not met. The trial enrolled the largest cohort of patients with HCC treated in this setting. Overall, 1114 patients were equitably randomized to take either sorafenib or placebo. The study did not met its primary and secondary endpoints since no differences were observed regarding recurrence-free survival (33.4 mo

vs 33.8 mo; HR = 0.94; 95%CI: 0.78-1.13, *P* = 0.26), time to recurrence (38.6 mo *vs* 35.8 mo; HR = 0.89, 95%CI: 0.73-1.08) and overall survival (not reached *vs* not reached, HR = 0.99, 95%CI: 0.76-1.30, *P* = 0.48). In this trial, a higher rate of sorafenib discontinuation due to drug-adverse events was observed compared to placebo (24% *vs* 7%)[190].

Additional studies have evaluated other targeted agents either in combination with sorafenib, or designed as head-to-head compared to sorafenib, or as second-line treatments following disease progression or inability to tolerate sorafenib; however, all these trials failed to demonstrate an improvement in overall survival[190].

CONCLUSION AND FUTURE DIRECTIONS

HCC is a difficult to treat and extremely complex malignant disease. Epidemiological data confirms an increasing number of new cases each year and this rise will persist due to the burden linked to HCV and obesity. Since the number of new HCC cases being diagnosed each year is nearly equal to the number of deaths from this cancer it is clear that the international scientific community and healthcare systems worldwide have no efficient answer to HCC. There are marked differences between countries in providing disparate quality of healthcare considering screening and surveillance programs; available treatment modalities and drugs; reimbursement of specific treatment options by the state-funded health insurance. In many countries worldwide liver transplantation is not a therapeutic option. In countries with national LT program the donor pool is a serious obstacle for treating more patients. Surveillance programs, so essential for the diagnosing an early stage HCC, are lacking in many countries. The experience from Japan clearly confirms importance of a successful surveillance program. Liver resection and TACE are the two treatment modalities offered to HCC patients even in underdeveloped countries. Since treatment allocation should be decided by a multidisciplinary board involving hepatologists, pathologists, radiologists, liver surgeons and oncologists guided by individualized-based medicine, HCC patients should be managed in high-volume, tertiary, university centers. This approach is important to achieve the best possible outcome from a variety of potentially useful therapies and for research purposes.

Different combination therapies tested in various studies failed to demonstrate a real benefit in terms of overall survival. This is mainly due to the complexity of the disease and due to the extremely heterogeneous patient populations included in clinical trials. The consensus conference on LT for HCC has shown that many controversies remained unanswered due to the lack of evidence. Therefore, high-quality randomized trials with better patient stratification are mandatory in the future to find patient populations that can benefit from certain treatment modalities. Basic research in HCC carcinogenesis is equally important. Combinations of different treatment modalities should be more exploited in order to improve survival and the quality of life of HCC patients.

In the management of HCC patients, several recommendations are important: (1) to establish a national surveillance program in as many countries as possible; (2) to further improve treatment modalities for patients on the waiting list for LT; (3) to improve the safety of liver resection and to reduce the recurrence rates following resection; (4) to investigate further and to upgrade results of the TACE treatment modality; (5) to continue research on novel molecular therapies; and (6) to continue research on novel molecular markers for better patient selection for various treatment modalities.

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REVIEW

Management before hepatectomy for hepatocellular carcinoma with cirrhosis

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Abstract

The global distribution of hepatocellular carcinoma (HCC) varies markedly among regions, and patients in East Asia and Central Africa account for about 80% of all cases. The risk factors are hepatitis B, hepatitis C, alcohol, and *etc.* The risk of carcinogenesis further increases with progression to hepatic cirrhosis in all liver disorders. Radical treatment of HCC by liver resection without causing liver failure has been established as a safe approach through selection of an appropriate range of resection of the damaged liver. This background indicates that both evaluation of hepatic functional reserve and measures against concomitant diseases such as thrombocytopenia accompanying portal hypertension, prevention of rupture of esophageal varices, reliable control of ascites, and improvement of hypoalbuminemia are important issues in liver resection in patients with hepatic cirrhosis. We review the latest information on perioperative management of liver resection in HCC patients with hepatic cirrhosis.

Key words: Hepatocellular carcinoma; Liver resection; Liver cirrhosis; Portal hypertension

Core tip: Radical treatment of hepatocellular carcinoma (HCC) by liver resection without causing liver failure has been established as a safe approach through selection of an appropriate range of resection of the damaged liver. This background indicates that both evaluation of hepatic functional reserve and measures against concomitant diseases such as thrombocytopenia accompanying portal hypertension, prevention of rupture of esophageal varices, reliable control of ascites, and improvement of hypoalbuminemia are important issues in liver resection in patients with hepatic cirrhosis. The latest information on perioperative management of liver resection in HCC patients with hepatic cirrhosis was reviewed.

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INTRODUCTION

The global distribution of hepatocellular carcinoma (HCC) varies markedly among regions, and patients in East Asia and Central Africa account for about 80% of all cases[1,2]. The risk factors are hepatitis B and aflatoxin in these regions[3], whereas hepatitis C and alcohol are risk factors in North America, Europe and Japan[4,5]. The risk of carcinogenesis further increases with progression to hepatic cirrhosis in all liver disorders. Hepatic cirrhosis is an irreversible pathological change and inhibition of disease progression has previously been considered difficult. However, advances in antiviral therapy now permit eradication or inhibition of replication of viruses[6].

Radical treatment of HCC by liver resection without causing liver failure has been established as a safe approach through selection of an appropriate range of resection of the damaged liver[7,8]. In the HCC practice guidelines of the Barcelona Clinic Liver Cancer (BCLC) staging system, liver resection is not recommended for patients with portal hypertension[9], and radiofrequency ablation (RFA) and transcatheter arterial chemoembolization are selected in many countries. In Japan, liver resection using appropriate preoperative management has been found to be safe and to improve the prognosis for patients with portal hypertension[10].

This background indicates that both evaluation of hepatic functional reserve[11] and measures against concomitant diseases such as thrombocytopenia accompanying portal hypertension, prevention of rupture of esophageal varices, reliable control of ascites, and improvement of hypoalbuminemia are important issues in liver resection in patients with hepatic cirrhosis[12]. In this report, we review the latest information on perioperative management of liver resection in HCC patients with hepatic cirrhosis.

DEFINITION OF HEPATIC CIRRHOSIS

Hepatic cirrhosis is the terminal stage of chronic liver disease, in which fibrous tissue accumulation due to necrotizing inflammatory reactions makes the liver surface rough and irregular[12]. Histologically, lobular structure remodeling and pseudolobule formation are observed; *i.e.*, hepatic cirrhosis is a morphologically defined disease[13].

Classification

Hepatic cirrhosis is classified based on: (1) cause; (2) function and clinical stage; and (3) node size-based morphology (World Health Organization classification). In (2), hepatic cirrhosis is classified into compensated and decompensated phases, and by the Child-Pugh classification, as described below. In (3), hepatic cirrhosis is classified into three types: micro-nodular type, with nodes < 3 mm, macro-nodular type, with nodes ⩾ 3 mm, and mixed nodular type, in which both nodules are mixed.

Cause

Persistent hepatitis virus B and C infections and excessive alcohol intake are the causes in many patients. The specific types are primary biliary hepatic cirrhosis; autoimmune hepatitis; non-alcoholic steatohepatitis; and metabolic (Wilson disease, hemochromatosis), congestive (Budd-Chiari syndrome), parasitic, and drug-induced types[12].

Diagnosis

Hepatic cirrhosis is definitively diagnosed by histological confirmation of lobular structure remodeling and pseudolobule formation on liver biopsy. However, liver biopsy is not optimal because performance of this procedure before liver resection has a risk of complications. Thus, it is desirable to evaluate the presence of hepatic cirrhosis based on blood chemistry and diagnostic imaging. Several formulas for this purpose using blood tests have been reported[14-17] (Table 1). The aspartate aminotransferase (AST) to platelet ratio index (APRI index) is based on the AST level and platelet count. The diagnostic performance for hepatic cirrhosis C using a cut-off of 1.0 is about 77% sensitivity and 75% specificity[16].

In imaging diagnosis, transient elastography (FibroScan™) can be used for noninvasive measurement of liver stiffness (stiffness), in which liver elasticity is determined by measuring the velocity of transmission in the liver of a single shear wave emitted from a specific probe of an ultrasonic diagnostic device[18,19]. A strong correlation between liver elasticity and fibrosis stage has been reported[20].

Staging

The most common hepatic cirrhosis classification is the Child-Pugh classification, in which 5 factors are scored: encephalopathy, ascites, serum bilirubin level, serum albumin level, and prothrombin activity[21,22]. However, diagnoses of encephalopathy and ascites are subjective, and evaluation of liver function is determined specifically at the time of the test, which are disadvantages in evaluation of hepatic functional reserve for liver resection. In planning for liver resection, the liver damage classification is more appropriate, particularly for HCC[23]. This classification uses the indocyanine green retention rate at 15 min (ICG-R_{15}), instead of encephalopathy in the Child-Pugh classification, and stricter measurements of serum albumin and prothrombin levels. This classification is particularly useful for preoperative selection of patients with favorable hepatic functional reserve[24].

The prognosis of HCC depends on the hepatic functional reserve and tumor stage. These variables are integrated in staging systems including the model for

Table 1 Prediction formula and discriminating factors for hepatic cirrhosis

Year	Ref.	Formula
2000	Ikeda *et al*[14]	Z = (0.124) × [γ globulin (%)] + (0.001) × hyaluronic acid (ng/mL) + (0.075) × platelet count (10^4/μL) + (-0.413) × gender (male = 1, female = 2) + (-2.005) The condition is hepatic cirrhosis when Z is positive, and chronic hepatitis when Z is negative
2007	Koda *et al*[15]	Fibroindex = (1.738) + (-0.064) × platelet count (10^4/μL) + (0.005) × AST (IU/L) + (0.463) × [γ globulin (g/dL)] The fibroindex value corresponds to fibrosis stage
2003	Wai *et al*[16]	APRI = 100 × [AST level/(upper limit of normal AST)/platelet count (× 10^9/L)]
2006	Sterling *et al*[17]	FIB-4 = [age × AST (U/L)]/[platelet count (× 10^9/L) × ALT (U/L)$^{1/2}$]

APRI: AST to platelet ratio index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; FIB-4: Fibroindex-4.

Table 2 Definitions of the cancer of the liver Italian program score[27]

Variable	Score		
	0	1	2
Child-Pugh stage	A	B	C
Tumor morphology	Uninodular and extension ⩽ 50%	Multinodular and extension ⩽ 50%	Massive or extension > 50%
AFP (ng/mL)	< 400	⩾ 400	
Portal vein thrombosis	No	Yes	

AFP: Alpha-fetoprotein.

Table 3 Definitions of the Japan integrated staging score[28]

Variable	0	1	2	3
Child-Pugh stage	A	B	C	
TNM stage[1]	I	II	III	IV

[1]By liver cancer study group of Japan.

end-stage liver disease[25], OKUDA[26], cancer of the liver Italian program (CLIP) (Table 2)[27], Japan integrated staging (JIS) score (Table 3)[28], modified-JIS score[29], and the Tokyo score[30], all of which are useful predictors of outcomes. Kudo *et al*[28] proposed the JIS score, in which the TNM stage and Child-Pugh classification are integrated. This score has advantages over the CLIP score (integration of the Child-Pugh classification, tumor morphology, alpha-fetoprotein, and portal vein tumor thrombosis) because (1) stratification of scores is distinct; (2) the prognosis of score-0 liver cancer is favorable; and (3) there is a definitive JIS score for cases with a poor prognosis. Integrated staging is useful for prediction of outcomes, but inappropriate for selection and comparison of treatment methods[31].

TREATMENT OF THROMBOCYTOPENIA

A reduced platelet count is an indicator of hepatic cirrhosis, and liver resection requires measures against thrombocytopenia to reduce the risk of hemorrhage[12]. Low preoperative platelet count is independently associated with increased major complications, post-operative liver insufficiency, and mortality after resection of HCC[32].

Partial splenic embolization is performed to improve hypersplenism through partially necrotizing the spleen by embolization of the splenic artery with a gelatin sponge or metal coil[33,34]. Long-term maintenance of the increased platelet count requires extensive splenic embolization of about 80% (splenic volumes ⩽ 700 mL)[35], but this treatment is accompanied by risks of complications such as abdominal pain (82.4%), fever (94.1%), and splenic abscess (1.2%)[36]. A short-term minimum effect of embolization is believed to be sufficient to prevent hemorrhage after liver resection[37].

Splenectomy reliably improves portal hypertension and hypersplenism. In HCC accompanied by hepatic cirrhosis, splenectomy improves the serum bilirubin, albumin, and prothrombin levels, and splenectomy performed before liver resection has a significant benefit[38]. In contrast, splenectomy before brain dead liver transplantation causes an increase in infection, decrease in survival rate, and high mortality[39,40]. Thus, it has been suggested that cases should be carefully selected for splenectomy. Also, since immune function is reduced in patients with hepatic cirrhosis, overwhelming post-splenectomy infection syndrome (OPSI) is a concern[41]. OPSI is a complication that develops rapidly regardless of the time after surgery and has a poor prognosis and high mortality (50%-70%)[42-44]. Pneumococcus is the causative bacteria in 80% of cases and a pneumococcus vaccine is recommended for splenectomized patients. Interferon administration following splenectomy may also induce OPSI; thus, antiviral therapy should be performed carefully. The incidences of portal vein thrombosis after splenectomy are 9%-29% and 1.6%-8.0% in patients with and without concomitant spleen enlargement, respectively[45-48]. Doppler ultrasonography and contrast CT are useful for early diagnosis of portal vein thrombosis following splenectomy. The timing of splenectomy varies among institutions (Table 4). Sugawara *et al*[49] recommended simultaneous splenectomy for readily resectable HCC

Table 4 Reports on liver resection and splenectomy for hepatocellular carcinoma complicated by hepatic cirrhosis accompanied by hypersplenism

Year	Ref.	Simultaneous splenectomy, 2-stage (No. of patients)	Platelet count ($\times 10^4/\mu L$)	Child-Pugh (A/B/C)	Mortality	Morbidity	Survival rate	Effect
1989	Takayama *et al*[38]	Simultaneous (12), 2-stage (8)	4.6	N	N	N	N	Expansion of indication of liver resection
1999	Lin *et al*[94]	Simultaneous (11)	5.2 ± 1.5	5/6/0	9.1%	27.3%	5 yr recurrence-free 66.7%	Improvement of serum bilirubin level
2000	Sugawara *et al*[49]	Simultaneous (35), 2-stage (13)	4.7 ± 0.3	N	0	47.9	3/5 yr survival rate: 72.3%/38.9%	Improvement of safety
2000	Shimada *et al*[95]	2-stage (6)	5.2 ± 1.5	1/4/1	0	17	N	Improvement of platelet count, albumin level, and Child classification
2003	Oh *et al*[96]	Simultaneous (12), no sp (6)	5.5 ± 1.5	10/8/0	11.1	66.7	N	Expansion of indication of liver resection
2004	Wu *et al*[97]	Simultaneous (41), no sp (485)	3.8 ± 2.1	419/85/23	1.5	20.5	N	Improvement of recurrence-free survival rate
2005	Chen *et al*[98]	Simultaneous (94), no sp (110)	6.2	125/79/0	N	15.2	5 yr survival 56%, recurrence-free survival 35%	Improvement of recurrence-free survival rate
2008	Sugimachi *et al*[99]	Simultaneous (4), no sp (11)	4.2 ± 0.8	9/6/0	6.7	47	N	3-yr survival rate equivalent to that after conventional liver resection
2015	Zhang *et al*[100]	Simultaneous (84), no sp (84)	6.1 ± 4.2	84/0/0	0	39.3	1/3/5 yr survival: 90%/78%/66%	Improvement of recurrence-free survival rate

N: Details unknown.

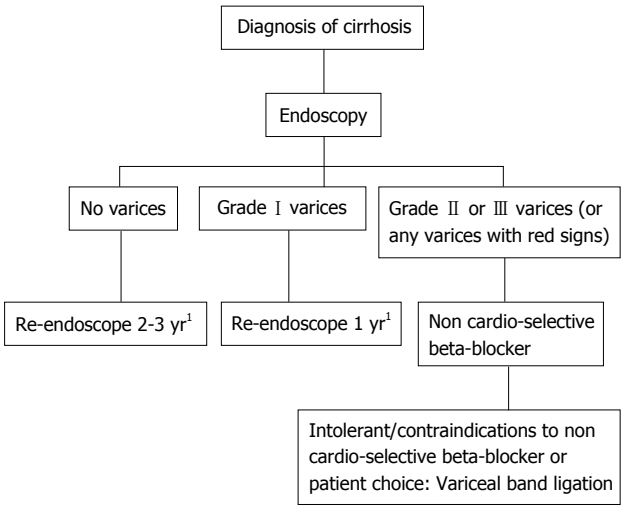


Figure 1 United Kingdom guidelines. Algorithm for surveillance of varices and primary prophylaxis in cirrhosis. [1]If there is clear evidence of disease progression this interval can be modified by clinician. Endoscopy should also be offered at time of decompensation[51].

in cases with favorable liver function and general conditions, and earlier splenectomy if these criteria are not met.

TREATMENT OF ESOPHAGEAL VARIX

The prognosis for patients with cirrhosis primarily depends on the occurrence of hemorrhage from esophageal varices and gastropathy. General rules for recording endoscopic findings of esophagogastric varices is formatted with location, form, color, red color signs, bleeding sings and mucosal findings (Table 5)[50]. According to the United Kingdom guide lines, esophageal varices are classified into 3 grades based on the size of varices[51]. Grade Ⅱ/Ⅲ varices (large) are indicated to beta-blocker or variceal ligation (Figure 1). McCormack classification is useful to definite of portal hypertensive gastropathy (Table 6)[52]. Thus, endoscopy should be performed before liver resection to avoid overlooking esophageal varices because the portal blood pressure rises after liver resection and this may aggravate varices. For patients with a history of hemorrhage from a varix, treatment of the varix before liver resection is required. For patients with a large (F2 or larger) varix accompanied by red color sign based on above general rules, preventive treatment is indicated[53].

Currently, endoscopic treatment is the standard for esophageal varices, using endoscopic injection sclerotherapy and endoscopic variceal ligation[54]. Balloon-occluded retrograde transvenous obliteration (BRTO) improves the varix and ICG-R_{15} value in patients with a gastric varix[55], but there is no evidence that BRTO improves the safety of liver resection. The endoscopic F factor (large varices) rating of bleeding esophageal varices can be a significant predictive factor for HCC[56]. So the screening of HCC is required after the treatment

Table 5 General rules for recording endoscopic findings of esophagogastric varices[50]

Category	Code subcategory
Location (L)	Ls: Locus superior
	Lm: Locus medialis
	Li: Locus inferior
	Lg-c: Adjacent to the cardiac orifice
	Lg-cf: Extension from the cardiac orifice to the fornix
	Lg-f: Isolated in the fornix
	Lg-b: Located in the gastric body
	Lg-a: Located in the gastric antrum
Form (F)	F0: No varicose appearance
	F1: Straight, small-caliber varices
	F2: Moderately enlarged, beady varices
	F3: Markedly enlarged, nodular or tumor-shaped varices
Color (C)	Cw: White varices
	Cb: Blue varices
	Cw-Th: Thrombosed white varices
	Cb-Th: Thrombosed blue varices
Red color signs (RC)	RWM: Red wale markings
	CRS: Cherry red spots
	HCS: Hematocystic spots
	Esophageal varices: RC0, RC1, RC2, RC3
	Gastric varices: RC0, RC1
	Te: Telangiectasia
Bleeding signs	Gushing bleeding
	Spurting bleeding
	Oozing bleeding
	Red plug
	White plug
Mucosal findings	E: Erosion
	Ul: Ulcer
	S: Scar

of large varices.

CONTROL OF ASCITES

Ascites accompanying hepatic cirrhosis involves interactions among various factors, including enhancement of liver lymph production with elevation of the portal blood pressure, enhancement of intra-abdominal portal permeability, reduction of the effective circulating blood volume, and enhancement of the sympathetic nervous system[12]. Treatment of fluid retention, which manifests as ascites, includes restriction of salts and water, administration of diuretics, and transfusion of an albumin preparation. If no effect is obtained in a short time, the patient is at high risk of liver or multiple organ failure, and liver resection should be avoided[57]. Selection of the smallest possible range of resection in patients with relatively favorable liver function and early resolution of ascites is the key to safe and successful liver resection[11].

IMPROVEMENT OF NUTRITIONAL STATUS

The association between preoperative sarcopenia and postoperative morbidity/mortality has been reported for various types of surgeries. Preoperative sarcopenia increased the morbidity rate including the rate of liver failure, in patients who underwent major hepatectomy with extrahepatic bile duct resection[58]. European Society for Parenteral and Enteral Nutrition guidelines recommended an energy intake of 35-40 kcal/kgBW per day (147-168 kJ/kgBW per day) and a protein intake of 1.2-1.5 g/kgBW per day for cirrhotic patients perioperatively[59].

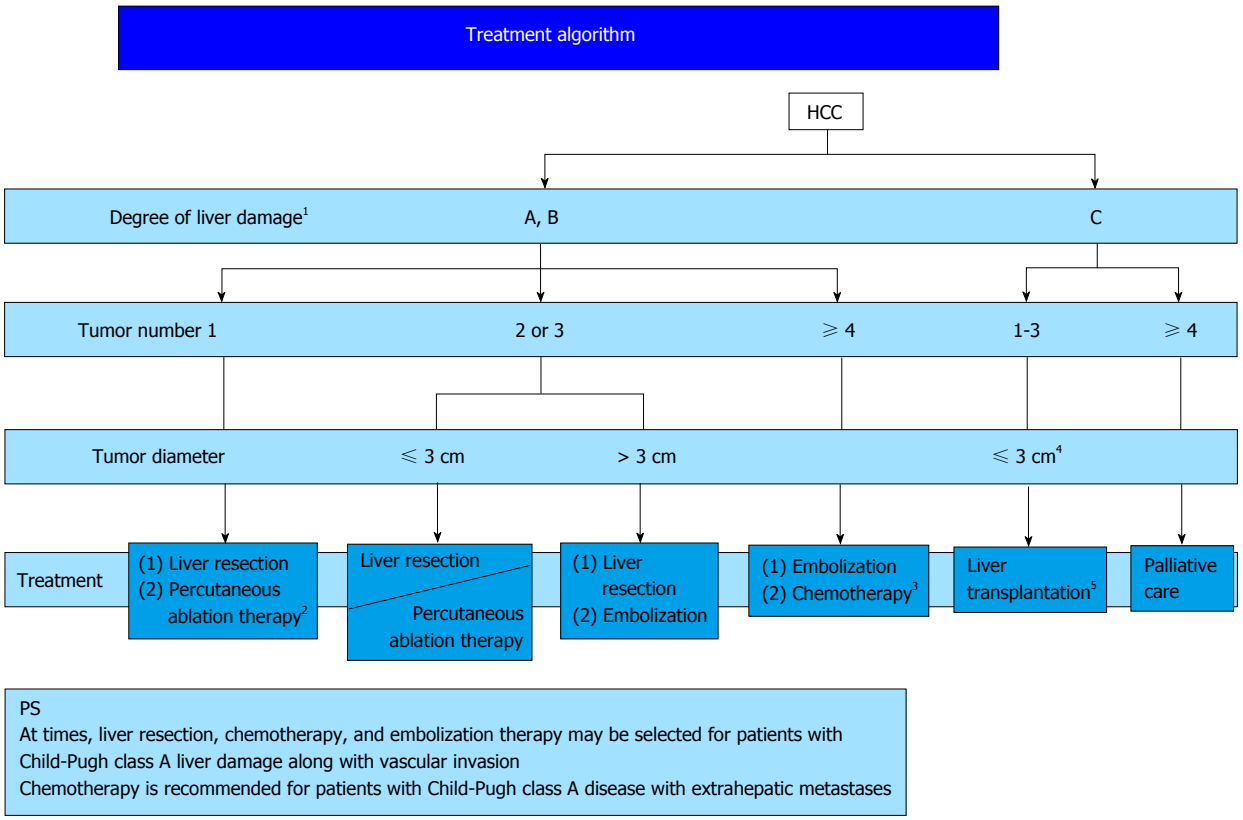
Protein-energy malnutrition (PEM) occurs in 27%-87% of patients with hepatic cirrhosis, and the level of branched-chain amino acids (BCAAs) is markedly reduced[60]. In PEM, hypoalbuminemia is observed and BCAAs are used for processing of ammonia and as an energy source for gluconeogenesis in skeletal muscle. In hepatic cirrhosis, serum albumin and plasma BCAA levels are positively correlated, and the prognosis is significantly poorer when serum albumin is < 3.5 g/dL[61,62]. Oral administration of BCAAs is of interest as a pharmacological and nutritional approach for improvement of hypoalbuminemia and insulin resistance, inhibition of angiogenesis, and activation of immune function[63]. In a randomized controlled trial (RCT) in 646 patients with decompensated hepatic cirrhosis who were divided into groups with and without treatment with oral BCAAs for 2 years, the incidences of death, liver cancer, rupture of esophageal varix, and liver failure were lower in the BCAA group and the prognosis was improved[64].

Table 6 McCormack classification for the presence of portal hypertension and portal hypertensive gastropathy[52]

Mild gastropathy	Fine pink speckling
	Superficial reddening
	Snakeskin (Mosaic-like) appearance
Severe gastropathy	Cherry-red spots
	Diffuse hemorrhagic lesion

SELECTION OF LIVER RESECTION RANGE

In Japan, East Asia and some European countries, ICG-R_{15} is used as an index of hepatic functional reserve. ICG-R_{15} is also a predictor of morbidity and mortality after surgery[65,66] and can be used to determine the acceptable liver resection range. In the therapeutic strategy for HCC, the BCLC staging system recommended by AASLD and EASL is used worldwide[9]. In Japan, the "treatment algorithm" described in the Clinical Guidelines for HCC is widely used to select the optimum treatment based on the liver function and tumor status (Figure 2)[67]. The Japanese treatment algorithm differs markedly from the BCLC system with regard to HCC with concomitant portal hypertension[68]. In the BCLC system, liver resection is not indicated if portal hypertension is present, and liver transplantation and RFA are recommended. In contrast, liver resection is recommended based on the ICG-R_{15} level in the Japanese treatment algorithm, and favorable outcomes



(Caution) 1: The Child-Pugh classification may also be used when non-surgical treatment is considered
2: Can be selected for tumors with a diameter of ⩽ 3 cm
3: Oral administration and/or hepatic arterial infusion are available
4: A single tumor ⩽ 5 cm or 2-3 tumors ⩽ 3 cm in diameter
5: Patients aged ⩽ 65 yr

Figure 2 Algorithm for treatment in Japanese hepatocellular carcinoma guidelines[67]. This algorithm has been simple and easy to memorize, consisting of three factors: (1) degree of liver damage; (2) number of tumors; and (3) tumor diameter. The recommendable treatment options are narrowed down to one or two by referring to this algorithm. HCC: Hepatocellular carcinoma.

have been reported[10].

Liver resection for HCC is chosen based on the balance between tumor status and liver function. Resection exceeding the hepatic functional reserve with the goal of cancer cure may lead to liver failure, whereas insufficient resection of the cancer due to excessive safety concerns may have a high risk of early recurrence. Therefore, it is important to select the optimum surgical procedure based on the tumor advancement and the acceptable liver resection range. Preoperative liver function can be evaluated using a galactose tolerance test, ^{99m}Tc-GSA liver scintigraphy, and an ICG tolerance test. The Makuuchi criteria are particularly useful for chronic hepatitis and hepatic cirrhosis cases (Figure 3)[11]. These criteria use the presence or absence of ascites, serum bilirubin level, and ICG-R$_{15}$ as evaluation items. Surgery is not indicated for cases with persistent ascites despite treatment with diuretics or if the serum bilirubin level is consistently > 2.0 mg/dL. The range of resection is determined based on ICG-R$_{15}$ in patients with a normal bilirubin level of ⩽ 1.0 mg/dL, *i.e.*, procedures can be selected for resection of up to 2/3 of the total liver volume (such as right lobectomy) in patients with normal ICG-R$_{15}$ (< 10%), up to 1/3 of the total liver volume (such as left lobectomy) for patients with ICG-R$_{15}$ of 10%-19%, and up to 1/6 of the total liver volume (Couinaud's segmentectomy) for patients with ICG-R$_{15}$ of 20%-29%. When ICG-R$_{15}$ exceeds 30%, surgery is limited to partial resection or enucleation. In a study in 1056 patients who underwent liver resection based on these criteria, the surgical mortality was 0%[8].

Systematic resection of cancer-containing regions perfused by branches of the portal vein should be performed within the range allowed by the liver function and with consideration of HCC invasion of the portal vein. Systematic subsegmentectomy of the liver was developed to overcome two contradictory goals: cancer curability and conservation of liver function[69]. Since HCC develops in a liver damaged by chronic hepatitis and hepatic cirrhosis in many cases, an insufficient volume of residual liver after major hepatectomy, such as lobectomy, may result in liver failure. To prevent liver failure, portal vein embolization (PE) is applied to the branch of the portal vein perfusing the planned region for resection to induce compensatory hypertrophy of the region remaining after liver resection[70]. PE is indicated for cases with ICG-R$_{15}$ < 10% and a ratio of the non-tumorous parenchymal volume of the resected liver to

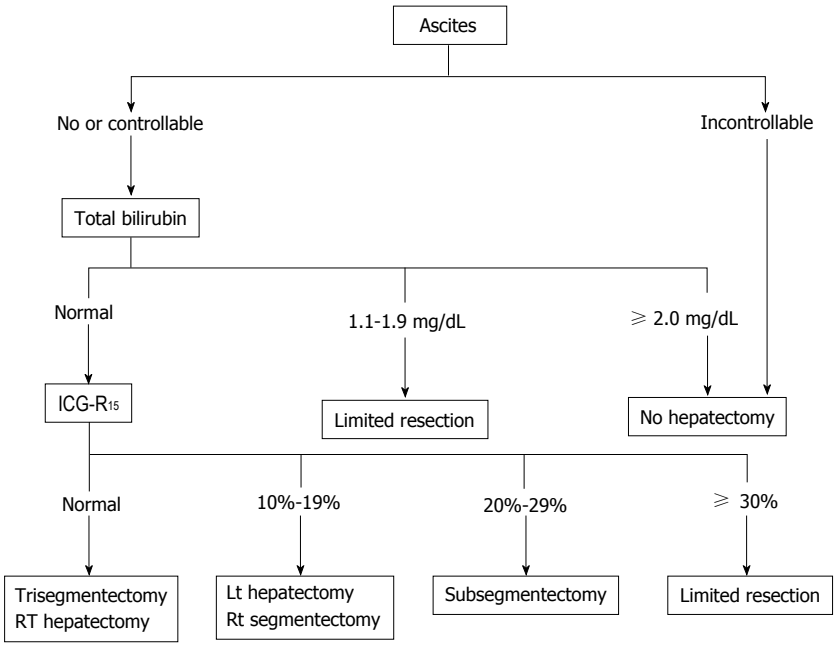


Figure 3 Makuuchi's criteria. Algorithm before proceeding to safety hepatectomy for hepatocellular carcinoma with cirrhotic liver. Makuuchi's criteria include three factors: ascites, total serum bilirubin, and the ICG-R_{15}: indocyanine green 15 min retention rate. This algorithm shows the maximal area for which an operation can be performed safely (modified ref.[11]).

that of the whole liver (R2) of ⩾ 60%, and for cases with ICG-R_{15} ⩾ 10% to < 20% and R2 of 40%-60%[71]. Both degree of liver hypertrophy and growth rate after PE are strong predictors of post-hepatectomy liver failure[72]. Recent introduction of 3-dimensional computed tomography (CT) has enabled simple and accurate determination of the positional relationship between the main vessels and the tumor, the range of resection, and measurement of the residual liver volume[73].

Since 1990, liver resection for HCC has been performed with acceptable blood loss at high-volume medical centers, and centers performing surgery with blood loss of about 500 mL have increased[74-77]. Blood transfusion may promote cancer recurrence and is likely to induce hyperbilirubinemia and liver failure[78]. Since a low hematocrit value is preferable for microcirculation of the liver, perioperative allogeneic transfusion should be avoided as much as possible in liver resection. Autologous blood transfusion is safe and useful for avoidance of allogeneic transfusion without increasing the risk of cancer recurrence[79]. Administration of fresh frozen plasma is recommended to supplement coagulation factors and maintain the effective plasma volume[80], but administration of fresh frozen plasma does not influence the course after liver resection and is not necessary if the serum albumin level 2 d after surgery is ⩾ 2.4 g/dL in Child-Pugh class A cases with intraoperative blood loss of < 1000 mL[81].

The immunosuppressed state after liver resection may lead to progression of liver failure and disseminated intravascular coagulation. In a RCT of steroid administration after liver resection, postoperative liver function was compared between groups treated with and without 500 mg/body hydrocortisone before liver resection. Serum bilirubin significantly decreased 2 d after surgery in the steroid group and there were significant differences in the time-courses of the bilirubin level and the prothrombin activity for 7 d after surgery. These results show the efficacy of steroid administration for liver resection[82].

POSTOPERATIVE ANTIVIRAL TREATMENT FOR HCC

HCC often recurs even after curative liver resection or RFA. It has been believed that controlling hepatitis and ameliorating the symptoms of cirrhosis prevent the recurrence of HCC. Several studies have examined the adjuvant therapies for their ability to prevent recurrence[83]. Eight RCTs were carried out to verify the efficacy of adjuvant interferon therapy for postoperative HCC[84-91]. It is suggested that adjuvant interferon-α reduced HCC recurrence and improved overall survival in patients with hepatitis C virus-infected HCC following curative treatment. The available evidence suggests that antivirus therapy with nucleoside analogs (lamivudine) should be recommended a postoperative preventive therapy for patients with hepatitis B virus-related HCC (> 500 copies of hepatitis B virus DNA/mL)[92,93].

CONCLUSION

Perioperative management is important in liver resection for patients with HCC and hepatic cirrhosis. New methods for evaluation and improvement of liver function are likely to facilitate expansion of the indication for liver resection.

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MINIREVIEWS

Fast track anesthesia for liver transplantation: Review of the current practice

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Abstract

Historically, patients undergoing liver transplantation were left intubated and extubated in the intensive care unit (ICU) after a period of recovery. Proponents of this practice argued that these patients were critically ill and need time to be properly optimized from a physiological and pain standpoint prior to extubation. Recently, there has been a growing movement toward early extubation in transplant centers worldwide. Initially fueled by research into early extubation following cardiac surgery, extubation in the operating room or soon after arrival to the ICU, has been shown to be safe with proper patient selection. Additionally, as experience at determining appropriate candidates has improved, some institutions have developed systems to allow select patients to bypass the ICU entirely and be admitted to the surgical ward after transplant. We discuss the history of early extubation and the arguments in favor and against fast track anesthesia. We also described our practice of fast track anesthesia at Mayo Clinic Florida, in which, we extubate approximately 60% of our patients in the operating room and send them to the surgical ward after a period of time in the post anesthesia recovery unit.

Key words: Liver transplant; Fast track anesthesia; Early extubation; Intensive care; Liver failure

Core tip: With proper patient selection, early extubation and bypassing of the intensive care unit is possible for patients undergoing liver transplantation. This needs a multidisciplinary approach and institutional support to be effective and can improve patient outcomes, as well as, improving resource utilization.

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INTRODUCTION

Historically, patients undergoing liver transplantation have been recovered in intensive care units (ICUs) following surgery to ensure a smooth transition through the recovery period. Advocates of this practice argue that these surgeries are associated with large fluid shifts and patients often have multiple significant comorbidities necessitating a slow, controlled emergence with close supervision[1-3]. However, as surgical and anesthetic techniques have improved, a growing number of centers worldwide have begun the practice of early extubation following transplantation. Some centers have even developed processes allowing select patients to completely bypass the ICU and be directly admitted to the surgical ward after transplantation. This article aims to discuss the history and techniques involved in fast track liver anesthesia.

LITERATURE SEARCH

We performed a PubMed and MEDLINE search using the terms fast track anesthesia, early extubation, liver transplant, abdominal transplantation, ICU, transplant anesthesia. Articles included original studies and review articles were reviewed for significance and additional articles were identified from the reviewed articles.

HISTORY

The idea of early extubation following major surgery was first described in cardiac anesthesia by Prakash *et al*[4] in 1977. In his study, Prakash found that 123 of 142 adult patients could spontaneously breath either immediately after or within 3 h following open-heart surgery. Furthermore, the group realized that the careful pre- and intra-operative assessment of potential candidates was needed to ensure success with this approach. More investigators followed, and as the literature supporting and refining the process of early extubation in cardiac anesthesia grew, anesthesiologists began exploring its application in liver transplantation (Table 1). Similar to the cardiac anesthesia experience, liver transplant teams began the arduous task of trying to determine which patients were most likely to succeed with this new approach. Rossaint *et al*[5], in 1990, suggested that patients who have been given minimal fluids may be good candidates. In their study, fluid was administered only when there was a fall in the cardiac index and ventricular filling pressures. This resulted in 5/36 patients being extubated immediately after surgery and an average time to extubation of 6 h for the remainder. Mandell and her team were successful at defining criteria for early extubation by examining patients that were successfully extubated within 8 h after surgery[6]. After deriving a list of extubation criteria and retrospectively comparing their success rates to another university program, they found that patients with good donor liver function, hemodynamic stability, an alveolar-arterial gradient of < 150 mmHg, and no encephalopathy tended to do well with immediate extubation. Criteria that did not significantly affect extubation were age > 50, United Network for Organ Sharing status 2-4, intraoperative transfusion requirements, and coexisting diseases. That same year, Neelakanta *et al*[7] published a paper describing the immediate extubation of 18 patients after liver transplantation followed by ICU admission. There were no incidents of reintubation. When the extubation group was retrospectively compared to matched controls, no differences in outcomes were discovered. Plevak *et al*[8] reported that an integrated plan encompassing all levels of care for the first 48 h after liver transplant reduced the time to extubation and shortened ICU stays without the need to change personnel and intraoperative protocols.

Over the next several years, researchers continued to refine which criteria best predicted success with early extubation. As the popularity with early extubation grew, transplant programs developed internal protocols to streamline the perioperative process and the percentages of patients given the opportunity for early extubation increased. Experience bred confidence, which in turn lead to more early extubations, better outcomes and improved patient selection. Biancofiore showed an increase in the extubation rate from an initial rate of 19% to 82% over 5 years as the anesthesia team became more confident with the process[1]. Similarly, the overall trend had been an increase in rates reported in the published studies from the same time era. Starting with success rates of 18.7% in 2001, multiple studies now describe rates closer to 90% as of 2010[9-12]. Today many centers around the world participate in early extubation of liver transplant patients, some have even progressed to bypassing the ICU altogether[2,13]. For the purpose of this paper, the process of bypassing the ICU and going directly from the postoperative care unit to the surgical ward is termed "fast tracking".

ARGUMENTS FOR AND AGAINST FAST TRACK ANESTHESIA

Proponents and advocates of fast track anesthesia have raised several points to support their arguments (Table 2). Early investigators believed that prolonged intubation following complex surgeries, such as liver transplant, allowed the patients to adequately "recovery" from the stress of surgery. Additionally, this period theoretically allowed the physicians caring for the patient in the postoperative phase to adequately optimize hemodynamic and pulmonary parameters prior to extubation and ideally improve outcomes. Advocates for early extubation have argued that it may be beneficial for the new graft to limit the exposure to mechanical ventilation. Kaisers *et al*[14] reported the deleterious effects of positive end expiratory pressure (PEEP) on liver graft hemodynamics. They found that a PEEP of

Table 1 Early extubation in adult liver transplant recipients

Ref.	Patients (*n*)	Anesthesia	Criteria for extubation	Findings
Rossaint *et al*[5]	5/39	Fentanyl infusion, methohexital infusion, thiopental, pancuronium	"According to established criteria" but not clearly delineated	
Mandell *et al*[3]	University of Colorado: 16/67 early extubation; UCSF: 25/106 early extubation	Thiopental, succinylcholine, isoflurane or desflurane, fentanyl, lorazepam, doxacurium	Preoperative: UNOS status 3 or 4; No coexistent disease; Age < 50 yr; No Encephalopathy; Intraoperative: Good donor liver function; < 10 units of red blood cells administered; No vasoactive support at end of surgery; A-a gradient < 150 mmHg	University of Colorado: 0/16 reintubations; UCSF: 2/25 reintubations (hypoventilation/ respiratory failure)
Neelakanta *et al*[7]	35 total patients: 18 extubated in OR; 17 extubated in ICU	Midazolam, thiopental, succinylcholine, isoflurane, fentanyl, morphine, pancuronium	Good nutritional status, no significant cardiac or pulmonary disease, uneventful surgical course, < 3 units of red blood cells transfused, sign of early graft function, normothermia. Decision was made by anesthesiologist after consultation with surgeon	O reintubations for either group. No difference in ICU length of stay; Immediate extubation group had more respiratory acidosis on admission to ICU
Biancofiore *et al*[1]	365 total patients: Group A: 211 extubated in OR; Group B: 113 extubated < 24 h; Group C: 41 extubated > 24 h	Fentanyl, thiopental, cisatracurium, sevoflurane, remifentanil	Awake, following commands, clinical evidence of neuromuscular reversal, normocarbia, respiratory rate < 25, adequate oxygenation (pulse oximetry > 95% with FiO_2 < 0.5), hemodynamic stability	Group A: 2/211 reintubations (surgical bleeding, pneumonia); Group C: 4/41 reintubations (surgical bleeding, pneumonia, hepatic artery thrombosis). Non-invasive ventilation performed in 11/211 Group A and 6/113 in Group B
Glanemann *et al*[9]	546 total patients: Group 1: 102 extubated in OR; Group 2: 383 extubated < 24 h; Group 3: 61 extubated > 24 h	Fentanyl, methohexital, pancuronium	Hemodynamic stability, normothermia, tidal volume of 5-8 mL/kg, respiratory rate < 20/ min, adequate minute ventilation, positive gag reflex, awake and responsive	Group 1: 9/102 reintubated; Group 2: 50/383 reintubated; Group 3: 22/61 reintubated; Survival at 5 yr greatly reduced in Group 3 and in patients whom underwent reintubation. Liver graft reperfusion injury significantly influenced success and time to extubation
Skurzak *et al*[10]	652 total patients: 575 extubation in OR; 77 nonextubated patients	Varied: Isoflurane or sevoflurane, fentanyl, remifentanil, sufentanil, pancuronim, atracurium, cis-atracurium. Extubated in OR	Conventional criteria used to determine for extubation. Contraindications to early extubation: active bleeding with a need for abdominal packing, preoperative mechanical ventilation, grade 4 encephalopathy, graft dysfunction (acidosis, persistent coagulopathy, hemodynamic instability)	30/575 reintubations within 48 h (surgical interventions, oversedation, pulmonary failure, pulmonary edema, cerebral ischemia, hepatic/renal failure)
Mandell *et al*[2]	147 total patients: 111 extubated in OR; 23 no attempt at extubation; 13 failed to meet extubation criteria	Thiopental, succinylcholine, isoflurane or desflurane, fentanyl, lorazepam, doxacurium	Awake, following commands, positive gag reflex, tidal volumes > 8 mL/kg, respiratory rate < 20/min, normocarbia, adequate neuromuscular reversal, hemodynamic stability	2/111 reintubations within 48 (portal vein thrombosis, oversedation)

UNOS: United Network for Organ Sharing; ICU: Intensive care unit; UCSF: University of California San Francisco.

Table 2 Arguments for/against fast track anesthesia

Pro	Con
Improved graft blood flow	Need for recovery after surgical stress
Decreased complications from mechanical ventilation	Time to optimize cardiopulmonary parameters
Patient comfort	Chance of failed extubation
Less chest radiographs	Absence of large prospective studies showing benefit
Improved resource utilization	Chance of reoperation
Cost containment	

10 mbar significantly reduced cardiac index, SvO2 and widened the arteriovenous oxygen content difference when measured with a pulmonary artery catheter placed percutaneously into the hepatic veins. It has been hypothesized that this may be due to retrograde blood accumulation in the liver circulation due to an increased backpressure transmitted from the pulmonary circulation[15]. Several small animal studies lend support to these claims[16,17]. More recently, however, these findings have been challenged. Saner observed that PEEP values up to 10 mbar produced no significant

change in hepatic arterial and venous flow as measured by Doppler in deceased donor rescue liver transplant and living donor liver transplant recipients[15,18]. Holland *et al*[19], examined patients undergoing cardiac surgery and requiring mechanical ventilation postoperatively. His group found that a PEEP of 10 mbar did not influence the disappearance of indocyanine green, a flow dependent marker of liver function. Nonetheless, while there is controversy about the effect of ventilation on hepatic blood flow and graft function, there is strong evidence that unnecessary mechanical ventilation is associated with several complications, including muscle deconditioning, tracheal injury, and pulmonary infections, the incidence of which can be lessened with early extubation[3,20,21].

Early extubation and fast track anesthesia has been shown to decrease the total cost for hospitalization by either reducing the length of intensive care or bypassing the ICU completely[6,13,22]. Taner *et al*[13], showed a reduction in total room charges, as well as, a decrease in the amount of chest radiographs and arterial blood sampling. This translates to better resource utilization and may be beneficial in areas with limited resources and in environments where cost containment is important[12].

Early extubation is not without significant risk, especially when dealing with patients possessing significant comorbidities. In a review of 11 studies by Wu *et al*[12], reintubation rates ranged from 3% to 35%. A variety of reasons including respiratory insufficiency, pneumonia, and reoperations were cited as common reasons for reintubation. Glanemann found an 11.7% reintubation rate among patients extubated in the operating room *vs* a 36% rate in patient extubated in the ICU[9]. Additionally, they found a higher incidence of tracheostomy in the ICU group. In the study, patients with acute liver failure, retransplantation, Child C status, and complicated surgeries requiring more than 6 units of packed red blood cells had an increased risk for prolonged postoperative mechanical ventilation[9].

ANESTHESIA FOR FAST TRACK

Most early studies have employed a balanced anesthetic approach[1,6,7,9-11,23,24]. This typically consisted of thiopental or propofol combined with opioids at induction, followed by inhalational agents and narcotics for maintenance. Concern has been raised over the use of propofol infusions for liver transplantation based on the fact that concentrations appear to increase during the anhepatic phase[25,26]. This may result in unpredictable levels and interfere with the ability to ensure a rapid emergence. The use of bispectral index monitoring may help offset this side effect by preventing overdosage[27-29].

Isoflurane, desflurane, and sevoflurane have been used in studies evaluating early extubation. Dose requirements for both desflurane and isoflurane have been shown to decrease during the anhepatic phase[30,31]. Increasing Model for End-stage Liver Disease (MELD) score also appears to be inversely proportional to volatile agent requirements[30]. These findings necessitate careful titration of these agents in patients planning to undergo early extubation and fast track anesthesia to prevent prolonged emergence.

Most often, neuromuscular blockade is achieved with atracurium or cis-atracurium, however vecuronium, rocuronium, and pancuronium have all been employed in studies evaluating early extubation[12]. As vecuronium, rocuronium, and pancuronium utilize hepatic metabolism to variable degrees, caution should be used when these medications. Delayed and primary graft nonfunction may result in prolonged neuromuscular block. Neuromuscular monitoring is an absolute requirement to ensure adequate return of muscle strength prior to extubation.

Adequate postoperative pain control without respiratory depression is a key component to anesthesia for fast track candidates, therefore an astute understanding of analgesic pharmacology in the care of the liver transplant patients is important. Liver transplantation recipients have reported decreased perioperative opioid requirements when compared to patients without liver disease undergoing other types of major abdominal surgeries, as the majority of opioid metabolism is liver-dependent. The severity of the liver disease and the process of the transplantation itself may alter the effects of different pain medications[32-34]. For example, when comparing healthy living liver donors undergoing graft procurement to patients with liver cirrhosis from chronic hepatitis B or C infection or hepatocellular carcinoma undergoing hepatectomy, the latter showed significantly lower morphine requirement on postoperative day 1[32]. Additional studies have shown that morphine usage was significantly less in patients undergoing liver transplant than in other liver operations, especially during the first three postoperative days[35-37]. Proper dosage of medications is crucial in achieving both adequate intraoperative anesthetic depth and postoperative pain control while avoiding over-sedation which increases the risk of prolonged postoperative mechanical ventilation.

At our institution, approximately 60% of 150 yearly liver transplant patients undergo fast track anesthesia and bypass the ICU completely. We typically induce anesthesia with propofol, fentanyl, midazolam, and succinylcholine. After intubation, anesthesia is maintained with isoflurane, fentanyl, and cis-atracurium. We limit our fentanyl dosage to 1000 micrograms unless the patient is opioid tolerant. If additional opioids are needed, we limit them to incremental dosing of up to 250 mg fentanyl aliquots. Postoperatively, patient-controlled hydromorphone is administered to manage incisional pain. Bispectral index monitoring is typically not used and our average operative time is 238 min for a primary liver transplantation. At the time of transplant, 30% have a raw MELD of 21-30 and 20% have a MELD of 31-40. Prior to extubation every attempt to ensure adequate hemostasis is attempted by the surgeon and anesthesia team using real time thromboelastrography and careful examination of the surgical field. Transfusion

goals are a stable hemoglobin of 8-10 mg/dL, an international normalization ratio of 1.5-2, a fibrinogen level greater that 170, and a platelet count of approximately 100.

We do not utilize set fast track criteria *per se*; rather all of the anesthesiologists base our determination on clinical experience after consultation with the operating surgeon. Typically redo transplants and patients requiring vasopressors or postoperative dialysis are admitted to the ICU after surgery. High volume transfusions are not an indication for ICU admission unless there is significant concomitant coagulopathy and a high likelihood of needing to transfuse more than 2 units of blood products per hour. Likewise, MELD score itself is not an indication for intensive care, although higher MELDs are more likely to be associated with significant comorbidities. We usually make our determination after graft reperfusion to give either the ICU or surgical ward time to prepare for the admission. On admission to the postoperative care unit, the anesthesia team evaluates relevant blood labs, an electrocardiogram, and a chest radiograph. After clearance from the anesthesia team, the patient is transferred to the surgical ward where the patient initially receives 1:1 nursing care for the first 24 h and further evaluation from the transplant hepatology team[13].

CONCLUSION

In summary, early extubation for large surgical cases started in cardiac surgery and is gaining popularity within the liver transplant anesthesia community. The practice of fast track anesthesia may decrease the incidence of pulmonary complications and improve graft function, and result in better resource management. As experience grows within our field, transplant teams have become better able to determine patients that can benefit from this practice[24]. Careful coordination and communication between the surgeons, anesthesiologists, and ward teams needs to be in place to ensure safe delivery of care.

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MINIREVIEWS

Progress in the treatment of pulmonary metastases after liver transplantation for hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and is the third leading cause of cancer-related death. Liver transplantation (LT) has become a curative treatment for patients with HCC. However, recurrence and metastasis after LT are the main factors reducing long-term survival in patients, and the lung is the most common site of metastasis after LT for HCC, although metastasis to liver, para-aortic lymph nodes and renal periphery are observed. Thus, the treatment of pulmonary metastases after LT for HCC has become a hot research topic, the successful treatment of pulmonary metastases can significantly prolong the survival of LT patients. Although single conventional treatment (chemotherapy, surgery and external beam radiation therapy), immunosuppression, image-guided minimally invasive therapy (radiofrequency ablation, microwave ablation, cryoablation, and brachytherapy) and molecular targeted drugs have had a significant effect, patients do not have durable remission and the long-term survival rate is disappointing. Therefore, improving existing treatments and identifying a more effective combination therapy are important research issues in the prevention and treatment of pulmonary metastases after LT for HCC. The paper reviewed single conventional treatments, new treatments, and combination therapy, to provide a basis for the best treatment of these patients.

Key words: Liver transplantation; Progress; Treatment; Pulmonary metastases; Hepatocellular carcinoma

Core tip: The treatment of pulmonary metastases after liver transplantation for hepatocellular carcinoma has become a hot research topic. Although single conventional treatment (chemotherapy, surgery and external beam radiation therapy), image-guided

minimally invasive therapy (radiofrequency ablation, microwave ablation, cryoablation, and brachytherapy) and molecular targeted drugs have had a significant effect, patients do not have durable remission and the long-term survival rate is disappointing. Therefore, we reviewed single conventional treatments, new treatments, and combination therapy, to provide a basis for the best treatment of these patients.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, is the third leading cause of cancer-related death, and the incidence is high and increasing[1,2]. Liver transplantation (LT) is carried out to treat HCC, especially in the early stage[3]. However, due to extrahepatic organ micrometastases, which cannot be found by imaging and cancer cells present in the blood circulation before LT, the characteristics of liver cancer (microvascular invasion, low differentiation, allelic imbalance, genetic diversity), the stage (super Milan criteria) and the administration of immunosuppressive agents during and after LT[4], result in a high incidence of postoperative recurrence and metastasis (60%-100%)[5]. Tumor recurrence and metastasis obviously decrease the chance of long-term survival after LT for HCC[6,7], thus recurrence and metastasis are the greatest obstacles to successful HCC treatment.

Lung is the most frequent site of metastasis after LT, although metastasis to liver, para-aortic lymph nodes and renal periphery are observed[8]. The successful treatment of pulmonary metastases can significantly prolong the survival of LT patients[9]. Therefore, improving existing treatments and identifying a more effective combination therapy have become important issues in pulmonary metastases after LT for HCC. The purpose of this paper is to provide an overview of therapies for pulmonary metastases after LT for HCC. Therefore, we reviewed single conventional treatments, new treatments, and combination therapy, to provide a basis for the best treatment of these patients.

CONVENTIONAL TREATMENT METHODS

Chemotherapy and external beam radiotherapy

The administration of chemotherapy for lung metastases after LT is controversial. The United Network for Organ Sharing data indicate that 48% of HCC patients received adjuvant chemotherapy after LT[10]. However, the results of therapy have been disappointing, and pulmonary metastases response rates after single or multiple agent chemotherapy regimens were low, with the 1-year survival rate between 0% and 30%[11]. Lee *et al*[12] reported that the most commonly used chemotherapeutic regimens were administered to patients following LT for HCC, and the median time to progression was 7.0 wk (95%CI: 5.8-8.2) and the median overall survival was 16.6 wk (95%CI: 10.1-23.1). Roxburgh *et al*[13] also found that HCC was generally chemoresistant and results using systemic therapy were disappointing. In addition, patients after LT required long-term oral immunosuppressive drugs, as the vast majority of patients could not tolerate chemotherapy toxicity, which was also a problem when administering chemotherapy[14].

There has been some progress in the use of external beam radiotherapy (EBRT), particularly the use of three-dimensional conformal radiotherapy and the tomotherapy system, which have significantly increased the partial or complete remission rate of lung metastases[15]. Jang *et al*[16] reported that 30-57.6 Gy of radiation for lung metastases resulted in a complete response rate of 26.3%, and the overall survival rate at 1 year was 50.1%. Matsui *et al*[17] also showed that stereotactic radiotherapy, performed at the site of tumor location two years after radiotherapy, resulted in survival of the patient without recurrence. However, at the time of diagnosis, most patients have multiple pulmonary or systemic metastases[18]. Therefore, the future trend in radiotherapy for pulmonary metastases after LT for HCC is to increase the tumor area dose by screening patients cautiously and using advanced radiotherapy positioning technology, while reducing the scope of normal tissue irradiated and the incidence of radiation toxicity. EBRT has significant value for lung metastases after LT for HCC.

Surgery

Surgery has been accepted as the first treatment for pulmonary metastases after LT for HCC for some time. Studies have confirmed that surgery is effective, and survival is reported to be between 24% and 78% at 3 years, with median survival ranging from 21 mo to 29 mo[19,20]. However, surgical resection of isolated metastasis following LT for HCC is limited to a few studies or case reports worldwide[21,22]. Bates *et al*[23] reported that five patients who had pulmonary resection of metastatic HCC after LT, had an average survival period of 44 mo after transplantation and 28 mo after pulmonary resection, these survival times were similar to those of patients following metastasectomy after liver resection for HCC. A year later, Zhang *et al*[18] also reported five patients who underwent standard lobectomy or wedge resection, and their survival ranged from 3 to 53 mo, with an average survival period of 18 mo. Similarly, Togashi *et al*[24] described two cases with long-term survival following pulmonary metastasectomy for HCC recurrence several months after living donor

liver transplantation, with no signs of further recurrence 2 years and 4 years 5 mo after resection of the lung metastasis. These studies show that resection of pulmonary metastases is an effective treatment method. However, growth of metastatic tumors in the alveoli causes no or mild respiratory symptoms in the early stages of disease. At the time of diagnosis, lung metastases have usually already developed into multiple metastatic lesions, and most patients are denied the chance of surgical treatment[25]. Furthermore, due to immunosuppression and surgical trauma, lung lesions may recur after resection at any time, and possibly metastases in other organs. The rate of recurrence and metastasis in patients receiving immunosuppressive therapy is significantly higher than in those who do not receive immunosuppressive therapy, indicating that immunosuppressive therapy plays a major role in tumor recurrence and metastasis after LT[26]. Therefore, the value of surgery in the treatment of lung metastasis after LT for HCC should be confirmed by further prospective multi-center clinical studies.

NEW TREATMENT METHODS

Immunosuppression

More and more studies have confirmed that immunosuppressants [*e.g.*, mammalian target of rapamycin inhibitors (m-TORi)] have anti-transplant rejection and multiple anti-tumor effects after LT for HCC[27,28]. Kawahara *et al*[29] found that m-TORi can decrease the risk of recurrence after LT for HCC and have lower drug toxicities. Cholongitas *et al*[30] also showed that patients on m-TORi had significantly lower recurrence rates following LT for HCC, thus m-TORi may represent an alternative immunosuppressive regimen with antineoplastic effects. Moreover, the early use of m-TORi can significantly prolong survival time and delay tumor progression after LT[31]. Klintmalm *et al*[32] indicated that m-TORi may have benefits in the oncology setting and in relation to HCV-related allograft fibrosis, metabolic syndrome, neurotoxicity, and survival time. However, clinical studies have demonstrated that immunosuppressive agents can cause serious adverse reactions in patients such as pneumonia and thrombocytopenia[33]. In patients with pulmonary metastases after LT for HCC, most were in poor physical condition and were unable to tolerate further treatment. Therefore, further research on reducing the side effects of m-TORi and controlling further progression with combination therapy for pulmonary metastases after LT for HCC, will have significant clinical value.

Minimally invasive therapy

In recent years, due to the development of medical imaging, many patients with pulmonary metastases may also be treated with minimally invasive treatments, such as interstitial laser coagulation, cryotherapy, microwave ablation, percutaneous ethanol injection, radiofrequency ablation (RFA), and ^{125}I brachytherapy[34,35]. RFA uses thermal and non-thermal effects which are generated by RFA electromagnetic waves in a biological medium to solidify cancer tissue, and the local temperature can be higher than 90 ℃, which kills tumor cells quickly and effectively. RFA has been demonstrated to be a safe and valuable treatment option and is accepted as the best therapeutic choice for patients with unresectable HCC pulmonary metastases. Lencioni *et al*[36] reported that RFA results in a high proportion of sustained complete responses in properly selected patients with pulmonary malignancies, and is associated with acceptable morbidity. Hiraki *et al*[37] found that RFA for 83 pulmonary metastases resulting from HCC was effective and safe in selected patients, where the effectiveness rate was 92% and survival rate was significantly improved. Therefore, RFA improves survival in patients with limited metastatic lung disease[38]. In addition, ^{125}I brachytherapy has also been used for pulmonary malignant tumors with good efficacy. Zhang *et al*[39] reported that computed tomography-guided ^{125}I seed implantation was safe and well tolerated for treating lung tumors, with few complications, and the 1-, 3-year, and median overall survival were 68.7%, 20.8% and 17.4 mo, respectively. While the minimally invasive treatment of malignant lung tumors has significantly progressed, there are few reports on pulmonary metastases after LT for HCC. With further clinical studies, coupled with the advantages of minimally invasive treatment, such as safety, ease of use, few complications and minimal trauma, this will become a new treatment for pulmonary metastases after LT for HCC.

Targeted drugs

Sorafenib is a small molecular multikinase inhibitor of vascular endothelial growth factor (VEGFR)-2, VEGFR-3, platelet-derived growth factor-b, raf, Flt-3 and c-KIT. Studies have demonstrated the ability of sorafenib to inhibit tumor proliferation *via* the RAS-/RAF-signaling pathway and angiogenesis[40,41]. Sorafenib has been approved for the treatment of advanced HCC[42]. Kudo *et al*[43] reported 15 cases with complete remission following treatment with sorafenib in patients with advanced metastatic HCC, including multiple liver lesions, lymph node metastases, adrenal metastases, lung metastases and vascular invasion, which were completely absent after treatment, and three tumor markers (AFP, PIVKA-Ⅱ and AFP-L3) returned to normal values. In addition, sorafenib has also made a breakthrough in the treatment of HCC recurrence after LT. Sposito *et al*[44] found that sorafenib was associated with an acceptable safety profile and had a survival benefit in HCC patients suffering recurrence after LT, the recurrence time was 38.1 mo, living conditions were significantly improved after cancer recurrence (the median survival from recurrence was 21.3 mo), and the only factor associated with survival after HCC recurrence in multivariate analysis was treatment with sorafenib (HR = 4.0; P = 0.0325). Yeganeh *et al*[45] in a large single-center retrospective study found that

sorafenib was safe and well tolerated in patients with recurrent HCC following LT and may be associated with a modest survival benefit, the rate of survival at 3, 6, 9, and 12 mo was 100%, 80%, 71% and 62%, respectively. However, studies on the therapeutic value of sorafenib in pulmonary metastases after LT for HCC are rare, and only a few case reports were identified in the present review. Furthermore, serious adverse reactions have been observed, and some patients could not tolerate conventional doses of sorafenib and required dose reduction, which led to progression and deterioration of the disease or death[14,46]. Therefore, further investigations are needed to confirm the efficacy of sorafenib in the prevention and treatment of HCC after LT in international and multi-center randomized studies with a large sample size. Nevertheless, sorafenib is still a safe treatment and has a place in the treatment of pulmonary metastases after LT for HCC.

Comprehensive treatments

In clinical practice, it is difficult to achieve the desired results in patients with HCC lung metastases using a single treatment, therefore it is necessary to combine two or even more treatment methods. At present, reports on the combination therapy of pulmonary metastases after LT for HCC are rare. Sakamoto *et al*[47] reported one patient with HCC, multiple lung metastases and peritoneal metastasis. The patient was treated with a variety of methods including injection of ethanol, chemotherapy, and surgical resection, and although the patient died from cerebral infarction caused by tumor thrombus, the patient's quality of life for five years was significantly improved. Matsui *et al*[17] described one patient with HCC who underwent right hepatic lobectomy and was found to have right lung metastases two years later, and lobectomy after chemotherapy was ineffective. The left lung was then found to have metastasis, the patient received directional EBRT, and quality of life within two years was good with no further recurrences. Li *et al*[48] reported 8 patients with HCC who underwent LT and then were found to have lung metastases. All patients received brachytherapy combined with sorafenib therapy. The local control rates of multiple lung metastases after LT for HCC after 4, 6, 12, 18 and 24 mo were 92.2%, 82.4%, 76.2%, 73.3% and 72.2%, respectively, and the overall 1-, 2- and 3-year survival rates were 100%, 50% and 12.5%, respectively. These studies suggest that treatment of patients with HCC metastases after LT should be individualized, with comprehensive therapies, or combined methods with a variety of treatments according to the condition of the patient, to improve their quality of life and survival.

CONCLUSION

Recurrence and metastasis are major problems restricting long-term survival after LT, while single treatments have had some success, they still need further study. New treatment methods have also had remarkable success and have the potential to delay progression and prolong survival, however, this does not apply to all patients. Therefore, improving the existing treatments, using individual and combination therapy, is the future direction for the prevention and treatment of pulmonary metastases after LT for HCC.

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