Serum Alpha-Fetoprotein as a Biomarker in Liver Transplantation

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Abstract

Alpha-fetoprotein (AFP) is one of the most widely tested biomarkers in medicine. It has long been used in surveillance and diagnosis of hepatocellular carcinoma (HCC), the second cause of cancer-related death worldwide. Modern imaging modalities have replaced AFP in screening and diagnosis of HCC in the last decade. However, the establishment of liver transplantation as the gold standard treatment for HCC patients brought AFP back to the focus of interest. AFP was thoroughly investigated as a selection criterion for transplant candidates and a potential predictor of posttransplant HCC recurrence and survival. The general conclusion that can be made from all the studies is that high or rising AFP values indicate aggressive tumor biology and correlate with poor differentiation, microvascular invasion, posttransplant HCC recurrence, and reduced survival. Different AFP cutoff values were proposed, with or without being incorporated into transplant selection criteria or prognostic models. Because of the wide range of the proposed AFP cutoff values (from 15 to 1000 ng/nL) and the major diversity of the suggested selection criteria and prognostic models, there is no universal agreement on a specific role for AFP in liver transplantation till now. Prospective validation of AFP roles in large well-conducted randomized trials needs to be performed to come up with definite conclusions that can be applied to clinical practice. Also, multiple new biological and genetic markers are being studied in surveillance and diagnosis of HCC with promising results.

Keywords

Alpha-fetoprotein • Hepatocellular carcinoma • Liver transplantation • Selection criteria • Prediction • Prognosis • Recurrence • Survival • Dropout • Down-staging

List of Abbreviations

AASLD	American Association for the Study of Liver Diseases
AFP	Alpha-fetoprotein
AFP-L3	Lens culinaris agglutinin-reactive alpha-fetoprotein
APASL	Asian Pacific Association for the Study of the Liver
CT	Computerized tomography
DCP	Des-gamma carboxyprothrombin
DeMELD	Dropout equivalent MELD
EASL	European Association for the Study of the Liver
HCC	Hepatocellular carcinoma
kDa:	Kilodalton
LRT	Locoregional treatment
MELD	Model for end-stage liver disease
mRECIST	Modified response evaluation criteria in solid tumors
MRI	Magnetic resonance imaging
NAS	Natural AFP slope
NCCN	National comprehensive cancer network
PEI	Percutaneous ethanol injection
PIVKA-II	Protein induced by vitamin K absence or deficiency
RFA	Radio-frequency ablation
TACE	Transarterial chemoembolization
TARE	Transarterial radio-embolization
TTD	Total tumor diameter
TTV	Total tumor volume
UCSF	University of California San Francisco
UNOS	United Network for Organ Sharing
US	Ultrasound

Key Facts About Serum Alpha-Fetoprotein (AFP)

- AFP is a glycoprotein that consists of a single polypeptide chain.
- AFP is the main plasma protein of the human fetus.
- AFP is synthesized in the liver and yolk sac of the human fetus.
- Expression of AFP gene in adults is abnormal and means a pathological condition
- An elevated AFP level in a patient with a liver mass does not necessarily mean the presence of an HCC.
- Up to 40% of patients with small HCC have normal AFP levels.
- AFP is no longer recommended as a diagnostic test for HCC in many of the recent guidelines.

- A high AFP level is a surrogate marker for microvascular invasion, poor differentiation, and aggressive HCC biology.

Definitions

Child-Pugh score	was originally developed to predict surgical outcomes in patients presenting with bleeding esophageal varices; it consists of five clinical features, each of them scored from 1 to 3 (albumin, bilirubin, prothrombin time prolongation, ascites, and encephalopathy) and is used to assess the degree of chronic liver disease and cirrhosis.
Hepatocellular carcinoma	is a primary malignant neoplasm derived from hepatocytes, accounting for about 80% of all liver cancers.
Liver cirrhosis	is a slowly progressing disease in which healthy liver tissue is replaced with fibrous tissue, leading to liver failure and multiple complications.
Loco-regional treatments for HCC	are local ablative therapies for treating patients with hepatocellular carcinoma (as percutaneous ethanol injection, thermal ablation, or intra-arterial chemo- or radio-embolization).
Model for end-stage liver disease (MELD)	is a score calculated using creatinine, bilirubin, and INR to measure the severity of liver disease. Initially, it is developed to predict 90 days mortality risk. Currently, it is used to prioritize recipients for cadaveric organ allocation.
Organ allocation	is the process of determining how organs are distributed; it should promote distribution of organs in an equitable, ethical, and medical sound manner.
Radio-frequency ablation (RFA)	is using the resistive heat resulting from medium frequency alternating electrical current delivered to the tumor through special needles to produce local hyperthermia and tissue destruction.
Screening	occurs when the patient is asymptomatic but undergoes testing in order to detect the disease early before development of symptoms.
Sensitivity of a clinical test	is the ability of the test to correctly identify those patients with the disease.
Specificity of a clinical test	is the ability of the test to correctly identify those patients with no disease.

Surveillance	is the process of serial application of the screening test to detect the disease before it becomes clinically evident.
Transarterial chemoembolization (TACE)	consists of placement of an intra-arterial catheter in the vessels supplying the tumor, to deliver high concentrations of a chemotherapeutic agent (e.g., doxorubicin) along with an embolic agent, such as lipiodol to achieve both targeted chemotherapy and deprivation of tumor arterial supply.

Introduction

Alpha-fetoprotein (AFP) is one of the most widely tested biomarkers in medicine. It has long been used for surveillance and diagnosis of hepatocellular carcinoma (HCC), in addition to several other pathological conditions. Recent advances in imaging technology, which enabled earlier detection and more accurate diagnosis of smaller HCC lesions, and establishment of liver transplantation as the gold standard treatment for selected HCC patients have both led to a switch of AFP utilization from a surveillance and diagnostic test for HCC to a selection and prognostic tool for liver transplant candidates with HCC. Researchers have been studying other HCC-related biomarkers including lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP), in addition to new genetic markers. However, the roles of these markers in clinical practice still need to be defined.

This chapter will review the changing role of AFP in management of HCC patients with a particular focus on the recent evidence related to its significance in the era of liver transplantation.

AFP Synthesis and Structure

Alpha-fetoprotein (AFP) is the main plasma protein found in the human fetus. It was first detected in the sera of human fetuses in 1956 by Bergstrand and Czar (Adinolfi et al. 1975). The liver and yolk sac are the two major sites for AFP synthesis during fetal development (Gitlin et al. 1972). AFP is believed to be the fetal type of serum Albumin as they are closely related both genetically and structurally, having extensive homologies in amino acid sequence (Deutsch 1991). The genes coding for both proteins have been localized to chromosome 4q (Harper and Dugaiczyk 1983). The AFP concentration in fetal serum is greatest at about 13 weeks of gestation and then starts to decrease while albumin synthesis increases. After birth, serum AFP concentration declines rapidly until it finally reaches the trace concentration found in

normal adults (<10 μg/L) usually by the end of the first year (Nikolic 1992). AFP is a glycoprotein with a molecular mass of about 64 kDa. It consists of a single polypeptide chain and contains 3.4% carbohydrate (Adinolfi et al. 1975).

Pathological Conditions with Elevated AFP

Normally, AFP gene is not expressed in adults except in certain pathological conditions, the most common of which are hepatocellular carcinoma (HCC) and germ-cell tumors as teratoblastoma or embryonal carcinoma of the testis or ovary. Patients with hepatic metastasis from gastrointestinal tumors as gastric, pancreatic, or colonic cancers may also have high AFP levels. A number of genetically determined disorders as hereditary tyrosinemia, ataxia-telangiectasia, and cystic fibrosis are associated with high AFP levels as well (Adinolfi et al. 1975).

Hepatocellular Carcinoma

Liver cancer (including HCC and intrahepatic cholangiocarcinoma) is the second cause of cancer-related death worldwide, and its incidence is still increasing (Bruix et al. 2015). The numbers of estimated new cases and estimated deaths because of liver cancer worldwide were almost similar in 2008, reflecting the high case fatality and aggressive nature of this disease (Jemal et al. 2011). HCC represents the main histological type of primary liver cancer – accounting for 70–85% of the total liver cancer burden – and 80% of those cases are due to chronic hepatitis B and C viral infection (Perz et al. 2006). Hepatitis C virus (HCV)-related HCC was recently reported to be the fastest rising cause of cancer-related death in the United States (El-Serag and Kanwal 2014). Other risk factors in western countries include alcohol-related cirrhosis and possibly nonalcoholic fatty liver disease (NAFLD) associated with obesity. Heavy exposure to aflatoxin B1, in parts of Africa and Asia, is another known risk factor (Jemal et al. 2011).

Surveillance and Diagnosis of HCC

Early detection of HCC allows potentially curative treatment options leading to better patient outcome and prolonged survival (Bruix et al. 2016; Fig. 1). So, surveillance protocols were developed for high-risk patient groups, including those with chronic viral hepatitis or liver cirrhosis related to viral infection. Previously, the majority of HCC patients had advanced disease at presentation, and their AFP levels were mostly elevated. AFP measurement, therefore, was not only used as a diagnostic test for HCC, but it was also implemented as a screening test in surveillance protocols for early detection of HCC (Gebo et al. 2002). In the absence of specific recommendations, regular follow-up of those patients by hepatic ultrasound and serum AFP used to be the most widely adopted surveillance protocol for HCC (Gebo et al. 2002). Recent

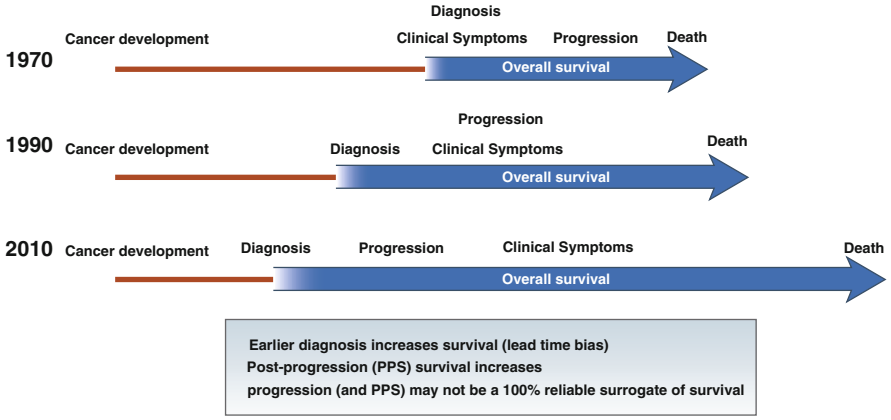

Fig. 1 Changes in diagnosis and treatment of HCC over time. Years ago, imaging techniques were not available. So, HCC was mostly diagnosed when symptomatic progression was close to death. Years later, HCC could be diagnosed at earlier stages, before symptoms developed. However, survival didn't improve much because of absence of effective treatment options. Nowadays, patients are diagnosed with early-stage disease due to surveillance programs, and the best therapy can be selected for each patient, increasing survival times (Modified with permission from Bruix et al. (2016))

studies, however, have shown that both the sensitivity and specificity of AFP are inadequate for effective surveillance and diagnosis of HCC (Lok et al. 2010).

AFP Sensitivity and HCC Surveillance

Up to 40% of patients with small HCC have normal AFP levels. The sensitivity of AFP in those patients would be only 60%, that is, obviously not sensitive enough for a screening test (Lok et al. 2010; Tateishi et al. 2008; Song et al. 2014). Also, measurement of AFP was found to provide no additional benefit to ultrasound surveillance, which showed significantly higher sensitivity for early HCC when performed every 6 months instead of annually (Singal et al. 2009). As such, recent guidelines of the National Comprehensive Cancer Network (NCCN) (Benson et al. 2014), the European Association for the Study of the Liver (EASL) (European Association for the Study of the Liver and European Organisation for Research and Treatment of Cancer 2012), and the American Association for the Study of Liver Diseases (AASLD) (Bruix et al. 2011) have all excluded AFP from HCC surveillance protocols and recommended HCC screening by hepatic US only every 6 months (Song et al. 2016). On the contrary, the Asian Pacific Association for the Study of the Liver (APASL) guidelines, the Chinese, and the Japanese guidelines still recommend the combination of AFP and US for HCC screening. Thus, exclusion of AFP measurement from surveillance of HCC is not universally agreed upon and may need to be further investigated (Song et al. 2016).

AFP Specificity and HCC Diagnosis

High AFP levels have long been considered diagnostic of HCC with high specificity. Recent data, however, suggest that its specificity – and consequently its value as a diagnostic test – is lower than what was previously believed (Tateishi et al. 2008; Bruix et al. 2011). Mild to moderate elevation of serum AFP occurs frequently in chronic liver disease, particularly chronic hepatitis C patients (Sterling et al. 2012). AFP can also be elevated in metastatic colorectal cancer (Sato et al. 1994), gastric carcinoma (Adachi et al. 2003), and intrahepatic cholangiocarcinoma, which is also more common in cirrhotics than in noncirrhotics (Bruix et al. 2011). Therefore, an elevated AFP level in a patient with a liver mass may be suggestive of – but does not necessarily mean – the presence of an HCC (Bruix et al. 2011). So, AFP is no longer recommended as a diagnostic test for HCC in many of the recent guidelines including the AASLD, EASL, and NCCN which rely mainly on the typical radiological features for diagnosis of HCC. AFP, however, remains an adjunctive tool for HCC diagnosis in Asian guidelines, as the APASL and Chinese guidelines (Song et al. 2016).

The typical contrast enhancement pattern in a four-phase dynamic cross-sectional study (enhancement in the arterial phase followed by washout in the portovenous or delayed phases) confirms the diagnosis of HCC (Bruix et al. 2011; Fig. 2). The latest AASLD guidelines recommend that a lesion smaller than 1 cm should be followed up closely at 3 months intervals using the same imaging modality that first showed the lesion. For lesions larger than 1 cm in diameter, further investigations should be performed with a four-phase dynamic study, either CT scan or MRI. If the appearance is not typical for HCC and does not suggest hemangioma, the other dynamic study should be performed or biopsy of the lesion is indicated (Bruix et al. 2011; Fig. 3).

Staging and Management of HCC

Most of the HCC patients have chronic liver disease, and a good percent of them will die from their failing liver rather than the HCC itself. This complicates the picture when considering treatment options. Proper staging is therefore crucial for successful management of HCC patients. The Barcelona Clinic Liver Cancer (BCLC) staging system has been repeatedly validated and widely adopted by most transplant centers (Fig. 4). It links prognosis and treatment options to the main variables that affect the patient's condition including cancer stage, severity of liver disease, performance status, and other comorbidities.

Liver Transplantation for HCC

Surgical resection and liver transplantation are the potentially curative treatment options for HCC (Agopian et al. 2015a). When indicated, liver transplantation is considered the gold standard treatment for patients having HCC and chronic liver disease as it is the only treatment that can address both the tumor and the liver disease

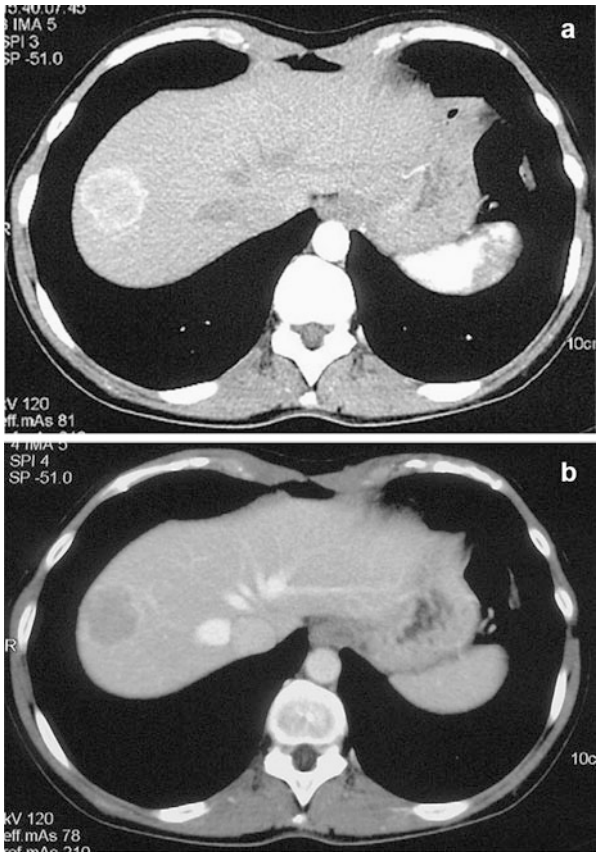

Fig. 2 Dynamic CT scan of a patient with HCC. Typical diagnostic pattern of HCC with contrast enhancement in the arterial phase (**a**) and washout in the portovenous phase (**b**) (Modified with permission from Golfieri et al. (2007))

at the same time (Hakeem et al. 2012). Any other treatment for HCC, even if complete tumor control is achievable, leaves the patient with the problem of a decompensating oncogenic diseased liver that will eventually fail and/or form new HCC lesions (Berry and Ioannou 2013). Liver transplantation for properly selected patients yields an excellent outcome, with more than 70% 5-year survival rates (Bruix et al. 2011).

Evolution of Transplant Indication and Candidate Selection for HCC Patients

The indication of liver transplantation in HCC patients has extremely evolved in less than two decades from being a contraindication in many centers in mid-1990s, until it became a priority deserving exceptional points under the model for end-stage liver disease (MELD) system for organ allocation in 2002 (Yao et al. 2007).

The outcome of early experience with liver transplantation for patients with HCC was generally poor with a 3-year survival rate of 31% (Bismuth et al. 1993). Bismuth et al. 1993 reported significantly better outcome with liver transplantation compared to

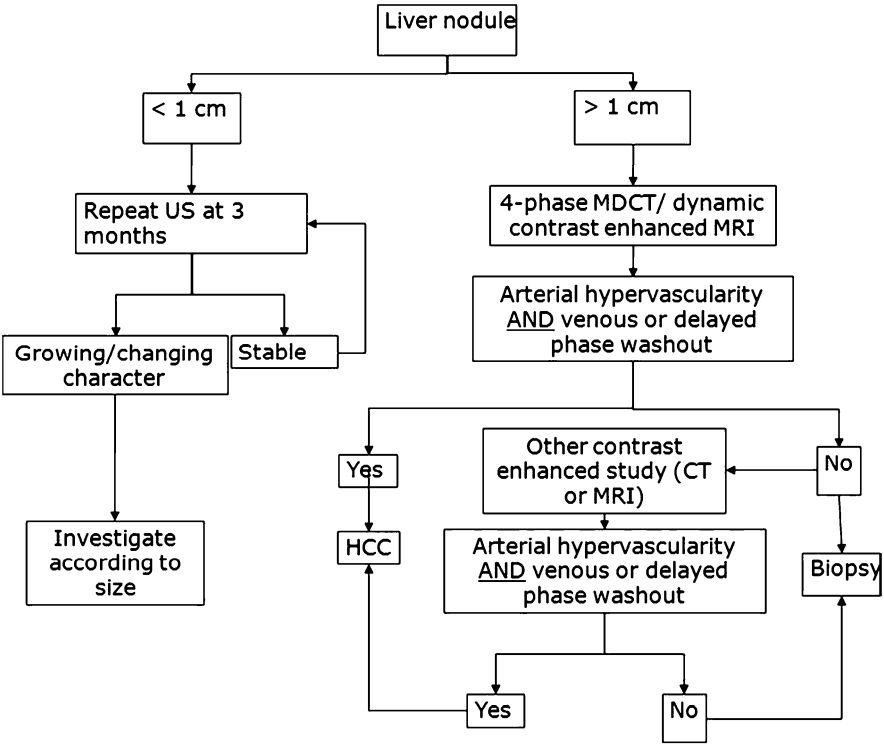

Fig. 3 Diagnostic algorithm for suspected HCC. *CT* computed tomography, *MDCT* multidetector CT, *MRI* magnetic resonance imaging, *US* ultrasound (Adopted with permission from Bruix et al. (2011))

surgical resection for HCC patients with limited tumor load, defined as single or double nodules less than 3 cm in diameter (Bismuth et al. 1993). The landmark study of Mazzaferro et al. (1996) reported excellent outcomes with liver transplantation for selected cirrhotic patients having small unresectable HCC lesions. Posttransplant survival of those patients equaled survival of non-HCC liver transplant recipients. Their selection criteria, known later as Milan criteria, included a single HCC nodule $\leq$5 cm in diameter or up to three tumor nodules; each is $\leq$3 cm with no evidence of macrovascular invasion or extrahepatic disease (Mazzaferro et al. 1996). Milan criteria not only got widely accepted and implemented by many transplant centers worldwide but also became the gold standard selection criteria for liver transplant candidates with HCC, to which any proposed modification needs to be compared (Mazzaferro et al. 2011).

Expansion of Transplant Selection Criteria for HCC

In spite of the general agreement on Milan criteria by many transplant centers across the world since its proposal till now, a number of studies suggested that Milan criteria could be very restrictive denying transplant access to many HCC patients

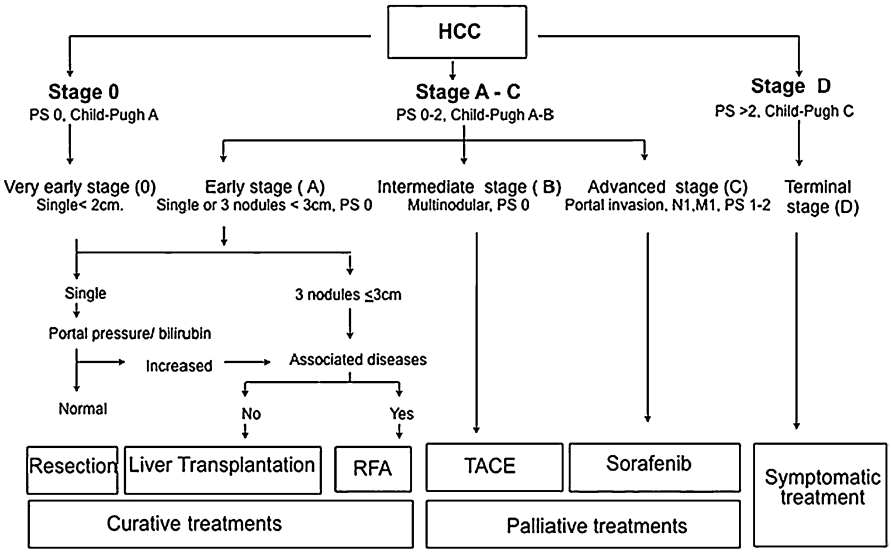

Fig. 4 The BCLC staging system for HCC. *M* metastasis classification, *N* node classification, *PS* performance status, *RFA* radiofrequency ablation, *TACE* transarterial chemoembolization (Adopted with permission from Bruix et al. (2011))

who may otherwise have a reasonably good chance of posttransplant survival (Llovet et al. 1999; Mazzaferro et al. 2009; Yao et al. 2001; Duvoux et al. 2012). Also, the estimated 10–15% under staging resulting from the less accurate imaging modalities at the time Milan criteria were proposed would allow some expansion of the morphologic criteria, having the currently available more precise radiologic scans (Bruix et al. 2011). Accordingly, several more permissive transplant selection criteria were proposed by different centers for HCC patients. Those proposed criteria suggested variable extensions of the morphological characteristics of the tumor, whether nodule number, maximum diameter, or both (Kashkoush et al. 2014). Among the extended selection criteria that were validated and accepted were the University of California, San Francisco (UCSF) (Yao et al. 2001) and the up-to-seven criteria (Mazzaferro et al. 2009). Multiple other criteria were proposed, but discussing them is not within the scope of this review.

UCSF Criteria

The UCSF criteria include patients with solitary tumors $\leq$6.5 cm, or those having three or fewer tumors, the largest $\leq$4.5 cm with total tumor diameter (TTD) $\leq$8 cm, and no macrovascular invasion (Yao et al. 2001). Excellent posttransplant outcome of HCC patients meeting those UCSF criteria was reported with 5-year survival of 75%. However, being retrospectively derived from explant pathology, UCSF criteria were considered cautiously until prospectively validated and showed similar

predictive ability for recurrence and survival compared to Milan criteria in spite of including up to 20% more candidates for transplantation (Yao et al. 2007).

Up-To-Seven Criteria

Mazzaferro et al. (2009) proposed other extended selection criteria known as the up-to-seven criteria where seven is the sum of the largest tumor size in cm and the number of tumors (Mazzaferro et al. 2009). The study included 1556 patients transplanted for HCC at 36 transplant centers (31 in Europe, 4 in the United States, and 1 in Asia). Patients outside Milan but meeting the up-to-seven criteria had 5-year posttransplant survival rate similar to patients within Milan if there was no evidence of microvascular invasion on explant pathology (71.2% vs. 73.3%) and a significantly lower 5-year survival (53.6%) if microvascular invasion was detected. Free online software called the Metroticket calculator was developed to calculate 3- and 5-year survival probability for potential candidates (Fig. 5). The calculator can also provide three survival estimates in relation to vascular invasion, whether present, absent, or unspecified. It is therefore clear that the major limitation of the model is the unavailability of information on microvascular invasion before transplantation. So, a surrogate preoperative, noninvasive marker of microvascular invasion is very crucial for implementing this model.

Fair Organ Allocation Mandates Reliable Prediction

Whether to adopt restrictive or permissive selection criteria depends mainly on availability of organs and dynamics on the transplant waiting list (Clavien et al. 2012). The imbalance between the number of potential liver transplant recipients and the available organ donors will continue to worsen (Berry and Ioannou 2013). This relative organ shortage necessitates optimization of the organ allocation process so that maximum benefit of the limited organ pool is achieved. A recent international consensus conference has recommended that: "Liver transplantation should be reserved for HCC patients who have a predicted 5-year survival comparable to non-HCC patients" (Clavien et al. 2012). It is generally accepted that HCC patients with an anticipated 5-year survival less than 50% should not be considered for liver transplantation (Zimmerman et al. 2008). To fairly apply this rule, reliable prognostic data are needed before declining patients' access to transplantation.

Tumor Biology: A Major Determinant of Treatment Outcome

Selection criteria depending on tumor morphology alone are generally crude predictors of outcome. While being the gold standard selection criteria, a number of patients exceeding Milan will have good prognosis if transplanted, and a subgroup of

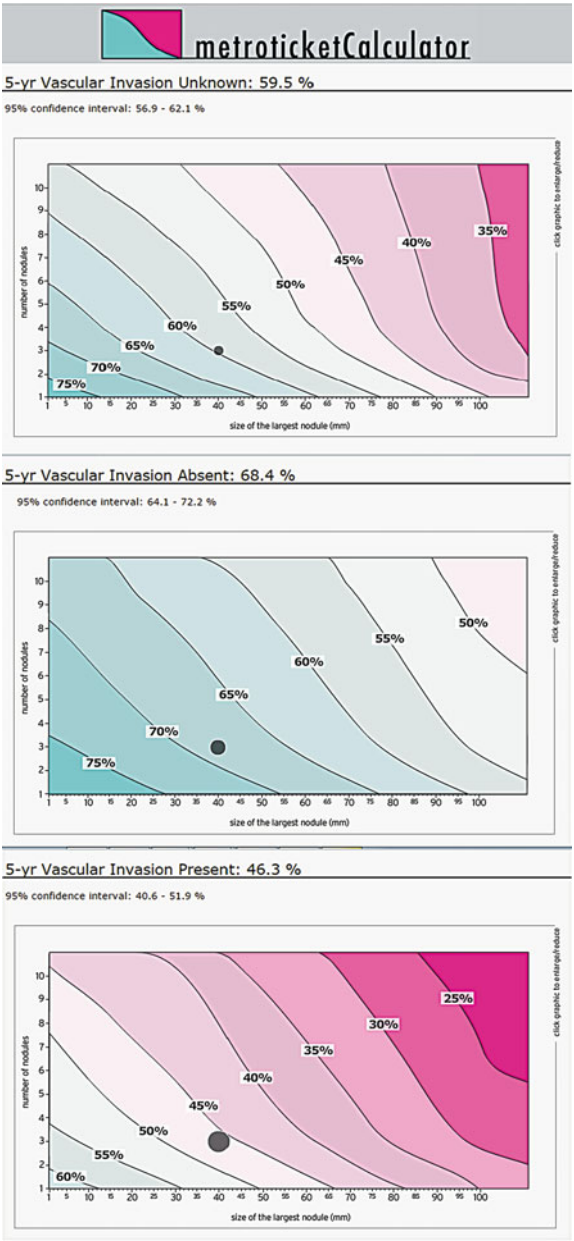

Fig. 5 Metroticket survival calculator for transplant candidates with HCC. An example of survival estimates generated by the free online Metroticket calculator for a patient with three HCC lesions; the largest is 4 cm in diameter. The calculator provides three survival estimates in relation to vascular invasion, whether unspecified (*upper*), absent (*middle*), or present (*lower*). This reflects the importance of AFP being a known surrogate marker for vascular invasion

patients within Milan will develop HCC recurrence and have poor prognosis (Hakeem et al. 2012; Duvoux et al. 2012). This could be explained by more than one fact. First, the accuracy of radiological tests will affect the predictive value of Milan and other related criteria that depend on morphological features of the tumor alone. Secondly, and more importantly, these criteria don't account for tumor biology, which is a major determinant of disease course and outcome (Clavien et al. 2012).

Features of aggressive tumor biology including microvascular invasion, high grade (poor differentiation), and satellite nodules, have been reported by many workers as independent predictors of HCC recurrence and poor survival following liver transplantation (Hakeem et al. 2012; Jonas et al. 2001; Moya et al. 2002; Duffy et al. 2007; Vibert et al. 2010). Those are microscopic features diagnosed only upon histological examination of explanted livers and cannot be definitely confirmed during the pretransplant stage (Gouw et al. 2011). Information related to tumor biology that can be obtained from a needle biopsy is usually incomplete, in addition to the documented risk of tumor seeding along the biopsy tract (Takamori et al. 2000). Therefore, presence of surrogate markers of aggressive tumor biology and poor histological features that can be measured preoperatively would be very valuable predictors of treatment outcome better than imaging-based selection criteria. AFP was thought to play that role, and it has been repeatedly studied in this regard. High-serum AFP expression correlates with extensive cellular proliferation, angiogenesis, and limited apoptosis (Song et al. 2016). AFP was found to be an independent predictor of both microvascular invasion and tumor differentiation; both of which are associated with disease recurrence and poor prognosis (Hakeem et al. 2012; Vibert et al. 2010).

AFP as a Predictor of HCC Recurrence and Survival After Liver Transplantation

AFP is the most widely tested biomarker for HCC (Lai et al. 2012a). A large number of studies have reported AFP significance as a predictor of disease recurrence and survival following liver transplantation for HCC (Hakeem et al. 2012; Clavien et al. 2012). Different AFP parameters were investigated including the highest absolute value, the latest value before transplant, and the dynamic AFP changes while on the waiting list. Other reports highlighted the significance of AFP as a predictor of patient dropout and/or success of pretransplant LRT. Also the response of AFP to locoregional treatment (LRT) and down-staging was investigated as a possible predictor of posttransplant outcome (Lai et al. 2013).

Significance of Absolute AFP Cutoff Values before Liver Transplantation

Several publications have reported different AFP cutoff values and their significant association with posttransplant HCC recurrence and poor survival. A recent systematic review and meta-analysis of the published studies has shown that AFP levels

>1000 ng/mL are associated with poor disease-free and overall survival, microvascular invasion, and poor differentiation. However, due to the heterogeneity of the included studies and the widely variable AFP cutoff values reported, which ranged from 20 to >1000 ng/mL, no valid statistical meta-analysis could be performed and no firm conclusions could be reached (Hakeem et al. 2012).

Hameed et al. 2014 reviewed 211 consecutive liver transplant recipients meeting Milan criteria, trying to establish an AFP cutoff for exclusion of candidates at high risk of HCC recurrence among this homogeneous group of patients (Hameed et al. 2014). The recorded AFP levels were the last ones within 3 months before liver transplantation. Vascular invasion was found to be the only significant variable predicting posttransplant HCC recurrence. However, AFP level >1000 ng/mL was the strongest pretransplant predictor of vascular invasion. The 5-year disease-free survival was 53% for patients with AFP levels >1000 ng/mL compared to 80% for patients with AFP levels ≤1000 ng/mL ($P = 0.026$). Interestingly, the significance of AFP cutoff >1000 ng/mL as a predictor of poor posttransplant outcome was reported by earlier studies from the same center. Proposing UCSF criteria, Yao et al. (2001) reported that an AFP level >1000 ng/mL was a predictor of reduced survival by univariate analysis only (Yao et al. 2001). Validating UCSF criteria, Yao et al. (2007) again reported the significance of AFP >1000 ng/mL as a predictor of HCC recurrence by both univariate and multivariate analyses (Yao et al. 2007). However, clinical application of this finding was not suggested at that time.

The AFP >1000 ng/mL cutoff was also reported by two large multicenter studies from Japan and France as a significant predictor of posttransplant HCC recurrence and poor survival (Duvoux et al. 2012; Todo et al. 2007). Both studies stratified the AFP cutoff into two values, the higher being >1000 ng/mL and the lower was either 200 (Todo et al. 2007) or 100 ng/mL (Duvoux et al. 2012). The 2 studies proposed new criteria/model for candidate selection (described later). The study of Todo et al. (2007) included 653 HCC patients who received living donor liver transplant at 49 transplant centers in Japan and reported significantly worse 1-, 3-, and 5-year overall survival of 64.9%, 42.5%, and 34% for patients with AFP levels ≥1000 ng/mL, compared to 83.8%, 77.3%, and 72.2% for patients with AFP ≤200 ng/mL (Todo et al. 2007). The study of Duvoux et al. involved 972 HCC patients transplanted at 21 centers in France and reported significantly higher 5-year HCC recurrence rate of 53% and worse 5-year overall survival of 39% for patients with AFP levels >1000 ng/mL compared to 16% and 67.5% for patients with AFP ≤100 ng/mL, respectively (Duvoux et al. 2012).

The significance of the AFP >1000 ng/mL cutoff value in relation to posttransplant tumor recurrence and poor survival was also reported by other workers (Lao et al. 2009; Zou et al. 2008). Zou et al. (2008) also showed an association between AFP levels >1000 ng/mL and early fatal recurrence of HCC within 1 year after liver transplantation (Zou et al. 2008).

Other lower absolute AFP cutoff values were suggested by different studies. An AFP cutoff value of 400 ng/mL was suggested by more than one researcher (Toso et al. 2009; Mailey et al. 2011; Merani et al. 2011) (see later). AFP >300 ng/mL was found among other variables to be significantly associated with HCC recurrence

after transplantation (Shetty et al. 2004). An AFP >200 ng/mL was reported by DuBay et al. (2011), to be significantly associated with dropout and lower posttransplant survival (DuBay et al. 2011).

Large AFP Studies Based on Transplant Registry Data

The huge database of the transplant registry of the United States, the United Network for Organ Sharing (UNOS), was analyzed by a number of researchers trying to define a specific role for AFP in liver transplantation for HCC. The conclusions of these studies drive their significance from being based on a very large sample of patients. The main disadvantage, however, is that data on HCC recurrence are missing with overall survival being reported instead (Hameed et al. 2014).

More than one study specifically recommended an AFP cutoff value of 400 ng/mL (Toso et al. 2009; Mailey et al. 2011; Merani et al. 2011) including the study of Toso et al. (2009) on 6478 transplant recipients that also proposed new selection criteria formed of AFP cutoff value of 400 ng/mL and total tumor volume (TTV) (Toso et al. 2009) (see later). Mailey et al. (2011) stratified the studied 2253 HCC transplant recipients into 3 AFP groups: low (<20 ng/mL), intermediate (20–399 ng/mL), and high (≥400 ng/mL). The intermediate and high AFP groups had significantly worse overall survival compared to the low AFP group, which was similar to that of patients with nonmalignant disease. Patients with AFP levels ≥400 ng/mL had significantly lower 1-, 3-, and 5-year overall survival of 82%, 63%, and 52% compared to 92%, 82%, and 74% for those with AFP <20 ng/mL ($P < 0.001$) (Mailey et al. 2011).

Merani et al. (2011) studied 6817 HCC patients and found that the last AFP level before transplantation was an independent predictor of overall survival ($p \leq 0.001$) and that an AFP ≤400 ng/mL was associated with favorable survival, irrespective of the original AFP level (even if originally >1000 ng/mL) (Merani et al. 2011).

A notable correlation between AFP and post-transplant survival was reported by the study of (Berry et al. 2013), which included 45,267 first-time liver transplant recipients and showed that progressive rise of pre-transplant AFP levels correlated with progressive worsening of post-transplant survival. Whereas patients without HCC had 6-year posttransplant survival of 72%, those with HCC had 6-year survival of 70% (if AFP was 0–5 ng/mL), 60% (if AFP was 16–65 ng/mL), 57% (if AFP was 66–320 ng/mL), or finally 51% (if AFP was >320 ng/mL). The study concluded that serum AFP, rather than tumor burden, was the preoperative variable most significantly associated with posttransplant survival. Moreover, patients exceeding Milan criteria had excellent posttransplant survival if their serum AFP levels were low (0–15 ng/mL), whereas those meeting Milan criteria had poor survival if their AFP levels were higher (≥66 ng/mL) (Berry and Ioannou 2013).

Dynamic AFP Changes Before Transplantation (The AFP Slope)

A number of studies investigated the possible significance of AFP dynamics before liver transplantation predicting posttransplant HCC recurrence and survival. Han et al. (2007) specifically studied AFP slope before liver transplantation in 48 patients transplanted for HCC (Han et al. 2007). They found that although the absolute value of AFP was not a predictor of recurrence, AFP slope was the only preoperative independent predictor of HCC recurrence. AFP slope also correlated significantly with large tumor diameter >7 cm and vascular invasion. Patients with a preoperative AFP slope >50 μg/L/month had significantly lower 1-year disease-free survival than those with an AFP slope ≤50 μg/L/month (40% vs. 90%, $P < 0.001$). The same center published a more recent study including 144 patients transplanted for HCC. They found that both rising natural AFP slope (NAS) (>0.1 μg/L/day) and Milan criteria were significant predictors of HCC recurrence. A rising NAS was also a significant predictor of microvascular invasion, a well-known risk factor for HCC recurrence (Dumitra et al. 2013).

The same concept was investigated by Vibert et al. (2010), where AFP progression was defined as an increase of serum AFP more than 15 μg/L/month. Significant 5-year overall and recurrence-free survival differences were found between patients with and without AFP progression (54% and 47% vs. 77% and 74% respectively, $P = 0.01$). AFP progression also significantly correlated with vascular invasion and satellite nodules on histological examination of the explanted livers. The study concluded that preoperative AFP progression >15 μg/L/month (equivalent to 15 ng/mL) was not only the most relevant prognostic factor to HCC recurrence and poor survival but also an independent predictor of unfavorable pathological features, which are themselves significant risk factors for HCC recurrence (Vibert et al. 2010).

Again, the AFP slope >15 ng/mL/month was reported by Lai et al. (2013) in a large multicenter study as a significant risk factor for posttransplant HCC recurrence and reduced survival, irrespective of the Milan status. The study included 422 HCC patients (306 within and 116 outside Milan criteria) who underwent LRT then liver transplant at six European centers between 1999 and 2010. In addition to the AFP slope, radiological progression of tumors according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) was found to be another significant predictor of HCC recurrence and death for all patients, whether within or outside Milan criteria (Lai et al. 2013). A controversial issue in calculating AFP slope was the two time points at which AFP was measured. Whereas the last pretransplant AFP value was clearly a significant point, it was not certain what is the best other time point to consider, given the fluctuations of AFP values with or without LRT. Therefore, the same group published a recent study investigating the AFP "delta-slope" (obtained by calculating the delta value between the two different AFP slopes, before and after LRT). The study retrospectively reviewed 124 candidates transplanted for HCC between 2004 and 2012 and reported that the AFP delta-

slope was also an independent predictor of posttransplant HCC recurrence and intent-to-treat survival. The 5-year intent-to-treat and recurrence-free survival rates were 66% and 92% versus 37% and 54%, for patients meeting and exceeding the AFP delta-slope cutoff value of 15 ng/mL/month, respectively (Lai et al. 2015).

Clearly, there is sufficient evidence to suggest that a rising AFP level before transplantation is a predictor of aggressive biology, posttransplant HCC recurrence, and poor outcome. However, the suggested AFP slope (and delta-slope) of 15 ng/mL/month still needs to be prospectively validated.

Transplant Selection Criteria Combining AFP and Tumor Morphology

Since serum AFP has emerged as a possible predictor of posttransplant survival in HCC patients, several studies have investigated combining it with different HCC morphologic criteria for better selection of transplant candidates in the current era of organ shortage (Berry and Ioannou 2013). The common objective of those studies is to optimize organ allocation so that candidates with expected better prognosis may get access to the limited organs. Even though most of those proposed combinations were shown to be comparable to Milan criteria, there is no universal agreement on any of them to replace Milan, which continues to be the gold standard selection criteria recommended by most guidelines.

AFP was combined with variable morphologic criteria including total tumor diameter (TTD), total tumor volume (TTV) or tumor number, and maximum size. Other studies have also combined AFP with Milan criteria, with or without other biomarkers.

Hangzhou Criteria

Zheng et al. 2008 retrospectively reviewed 195 HCC patients transplanted in China and proposed combined selection criteria (Hangzhou criteria), which consist of either (A) TTD $\leq$8 cm or (B) TTD >8 cm, with AFP $\leq$400 ng/mL and histopathologic grade I or II. The Hangzhou criteria included 27 more patients with no significant survival difference compared to Milan criteria (5-year survival 78% vs. 72%, respectively, $P > 0.05$) (Zheng et al. 2008). Recently, the same transplant center reviewed data of 6012 HCC patients transplanted in China and stratified Hangzhou criteria into type (A) (TTD $\leq$8 cm, or >8 cm but AFP $\leq$100 ng/mL with well to moderate tumor differentiation) and type (B) (TTD >8 cm but AFP between 100 and 400 ng/mL with well to moderate tumor differentiation) (Xu et al. 2015). Type A showed significantly higher 5-year recurrence-free survival rates compared to type B (69.5% vs. 39% for types A and B, respectively, $P < 0.001$). Since the main difference between types A and B is the AFP level, it is clear that high AFP is a significant risk factor for posttransplant HCC recurrence and poor survival.

Limitations of Hangzhou criteria include being based on Chinese population, where hepatitis B is the most prevalent cause of chronic liver disease and HCC complicating those patients tends to have better differentiation than HCC complicating hepatitis C infection (Busuttil 2008). Also the need for tumor biopsy in those criteria is a concern with the potential risks of seeding and/or bleeding (Lai et al. 2012b).

The AFP-TTD Criteria

A recent multicenter study from Italy proposed combined selection criteria more or less similar to Hangzhou criteria, without the need for tumor biopsy. (Lai et al. 2012b) retrospectively reviewed 158 HCC patients transplanted between 1999 and 2008 at the three centers of the Rome Inter-University Consortium for Organ Transplantation. AFP >400 ng/mL and TTD >8 cm were the strongest preoperative predictors of HCC recurrence. Combined AFP-TTD criteria were thus proposed (TTD ≤8 cm and AFP ≤400 ng/mL), which included 22% more candidates than Milan criteria (143 vs. 117 patients) and 7.5% more candidates than UCSF criteria (133 patients). Five-year disease-free survival rates were similar for patients meeting AFP-TTD, Milan, and UCSF criteria (74.4%, 72.9%, and 71.7%, respectively), with no statistically significant differences (Lai et al. 2012b).

The AFP-TTV Criteria

Instead of the TTD, Toso et al. (2009) introduced the principle of total tumor volume (TTV) and proposed composite selection criteria formed of TTV ≤115 cm^3 and AFP ≤400 ng/mL (Toso et al. 2009). As mentioned before, the study was a retrospective analysis of the large transplant registry database of the United States including 6478 recipients. Patients not meeting those criteria had an overall survival below 50% at 3 years.

The devised scoring system of Yang et al. (2007) is an example of combined transplant selection criteria formed of pretransplant AFP level, tumor size, and number (Yang et al. 2007). The study retrospectively analyzed data of 63 HCC patients who received living donor liver transplants in Seoul, Korea, between 1999 and 2005. The score of each parameter ranged from 1–4 points as follows: tumor size, ≤3, 3.1–5, 5.1–6.5, >6.5 cm; tumor number, 1, 2 or 3, 4 or 5, or ≥6 nodules; and AFP, <20, 20.1–200, 200.1–1000, >1000 ng/mL. Applying this scoring system, patients with a score of 3–6 qualify for transplantation and those with a score 7–12 won't. Milan, UCSF, and the proposed scoring criteria all significantly correlated with HCC recurrence and death with similar 3-year survival (80%, 78%, and 79%, respectively). However, the scoring criteria would include more candidates (70% compared to 65% for UCSF and 59% for Milan) while maintaining excellent posttransplant survival.

Table 1 Simplified user-friendly version of AFP model. The score is calculated by adding the individual points for each variable. A score of >2 identifies patients at higher risk of HCC recurrence (Modified with permission from Duvoux et al. (2012))

Variables	Points
Largest diameter, cm	
≤3	0
3–6	1
>6	4
Number of nodules	
1–3	0
≥4	2
AFP level, ng/mL	
≤100	0
100–1000	2
>1000	3

The AFP Model

The AFP model proposed by Duvoux et al. (2012) is another example of transplant selection criteria incorporating AFP with tumor size and number. The model was developed in a large multicenter study in France including 972 adult transplant recipients for HCC divided into two cohorts: training and validation (537 and 435 patients, respectively). The study showed that AFP is an independent predictor of HCC recurrence and it correlates with vascular invasion and tumor differentiation. The proposed model was reported to improve prediction of HCC recurrence and survival compared to Milan criteria. Using a simplified version of the model (Fig. 4), a score is calculated between 0 and 9. A score higher than 2 predicted markedly increased 5-year HCC recurrence risk (50.6% vs. 8.8%, $P < .001$) and decreased survival (47.5% vs. 67.8%, $P < .002$) irrespective of Milan status. A group of patients exceeding Milan had a score of 2 or lower because AFP was <100 ng/mL and had low 5-year HCC recurrence risk (14.4% vs. 47.6% $P = .006$). On the contrary, another group of patients within Milan had a score higher than 2 because AFP was >1000 ng/mL and had a high HCC recurrence risk (37.1% vs. 13.3%; $P < .001$) (Duvoux et al. 2012).

This AFP model was recently validated in a study from Spain including 109 patients transplanted for HCC (Varona et al. 2015). The study showed that the AFP model was a more accurate prognostic tool than other established criteria (including Milan and the up-to-seven criteria) in prediction of HCC recurrence and survival after liver transplantation (Table 1).

Incorporating AFP into Milan Criteria

As mentioned before, high serum AFP >1000 ng/mL was shown by more than one study to be a surrogate marker for vascular invasion and a significant predictor of posttransplant HCC recurrence. Hameed et al. (2014) found that incorporating AFP >1000 ng/mL as an exclusion criterion for patients within Milan would have excluded only 4.7% of patients from liver transplantation, but this could have

reduced HCC recurrence by 20% as well (Hameed et al. 2014). Also, Chaiteerakij et al. (2015) studied AFP and other biomarkers – including DCP and AFP-L 3% – and the value of combining these markers with Milan criteria for optimizing the liver transplant eligibility decision. They found that all biomarkers significantly correlated with HCC recurrence and that the hazard ratio for HCC recurrence was 3.5 for DCP $\geq$7.5 ng/mL and 2.8 for AFP $\geq$250 ng/mL. Combining Milan criteria with the selected AFP cutoff value of $\geq$250 ng/mL significantly increased the hazard ratio for HCC recurrence from 2.6 for being outside Milan criteria alone to 8.6 for the combined Milan/AFP criteria ($P < 0.001$) (Chaiteerakij et al. 2015).

Thus, incorporating pretransplant AFP into Milan criteria may exclude more candidates from liver transplantation, yet it improves the performance of Milan criteria in prediction of HCC recurrence after transplantation. Again, the appropriate cutoff value needs to be determined.

Combining AFP and DCP for Prediction of Transplant Outcome

Since the combination of AFP and DCP (PIVKA II) was reported to increase the sensitivity and specificity for diagnosis of HCC (Yang et al. 2007), it was also investigated as a better prognosticator of transplant outcome for candidates with HCC, either alone or with tumor morphologic criteria.

The A-P Levels

Combined criteria formed of both AFP $\leq$200 ng/mL and PIVKA II $\leq$100 mAU/mL were proposed by Todo et al. (2007) in a large multicenter study that included 653 HCC patients transplanted at 49 centers in Japan between 1989 and 2005. Both pretransplant AFP and PIVKA II (together, referred to as the A-P levels) were found to be independent predictors of HCC recurrence and poor survival by multivariate analysis. Patients within Milan criteria had no significantly different 5-year disease-free survival if they met or exceeded the A-P levels (96.4% vs. 74.7%). Patients beyond Milan criteria but within the A-P levels had favorable 5-year disease-free survival which was significantly better than that of patients beyond both criteria (79% vs. 40%). The combined A-P levels could include about 50% of candidates exceeding Milan criteria while maintaining satisfactory recurrence-free survival (Todo et al. 2007).

Combined AFP, DCP, and Tokyo Criteria

A similar and more recent study from Japan (Shindoh et al. 2014) proposed a combination of the serum AFP and DCP levels with the morphologic Tokyo criteria ($\leq$5 tumors with each tumor $\leq$5 cm). The study reported that presence of at least two of the following three factors including AFP >250 ng/mL and DCP >450 ng/mL and exceeding Tokyo criteria significantly correlated with a worse 5-year disease-

free and overall survival rates (20% vs. 97% and 20% vs. 84%, respectively, $P < 0.001$).

Dropout of Transplant Candidates and Predictive Role of AFP

HCC patients on the transplant waiting list may experience tumor growth beyond the acceptable criteria for transplantation leading to their "dropout" (disqualification for transplantation) (Cucchetti et al. 2011a). Longer waiting time on the transplant list – because of organ shortage – has resulted in overall increase of the dropout rates. The reported dropout risk of HCC patients ranged from 15% to 30% at 1 year (Galuppo et al. 2013). Llovet et al. (1999) compared outcomes of resection and transplantation for HCC and showed that dropout of transplant candidates as a result of long time on the waiting list was the cause behind the lower outcome of the transplant group on an intent-to-treat basis (Llovet et al. 1999). Therefore, prediction of patients who will likely dropout and their prioritization for liver transplantation is crucial for improving transplant outcome.

Interestingly, the same study (Llovet et al. 1999) and a number of other studies have shown that a higher AFP level is an independent predictor of patients' dropout while on the transplant waiting list (Yamashiki et al. 2004; Freeman et al. 2006; Majno et al. 2011; Washburn et al. 2010; Mehta et al. 2013). Yamashiki et al. (2004) found that a baseline AFP $\geq$100 ng/mL was the only factor that significantly correlated with delisting of transplant candidates due to tumor progression (Yamashiki et al. 2004). Washburn et al. (2010) reported a higher dropout rate of 24.9% in patients with AFP >1000 ng/mL which was significantly different from the 7.4% dropout rate of patients with AFP <500 ng/mL (Washburn et al. 2010).

AFP and Dropout Probability Scores

The scoring system called model for end-stage liver disease (MELD) is being used by transplant centers in the United States and elsewhere for prioritization of patients for liver transplantation. Because of the risk of dropout due to tumor progression, HCC patients are currently given exception MELD points to equate their access with non-HCC patients to donor livers (Washburn et al. 2010). Freeman et al. (2006) analyzed risk factors for dropout of candidates with HCC and proposed a dropout risk model by incorporating AFP level and maximum tumor diameter with the calculated MELD score. The proposed HCC-MELD score could predict dropout risk of HCC patients within 90 days of listing, and so patients with higher dropout risk may be prioritized for transplantation (Freeman et al. 2006).

Washburn et al. (2010) compared dropout rates and risk factors for HCC and non-HCC patients and found that – with the exception MELD points – the dropout rate of non-HCC patients was significantly higher than HCC patients ($P < 0.0001$). The study recommended that a scoring model incorporating MELD, AFP, and tumor

size would help prioritize HCC patients with higher risk of dropout and – at the same time – ensure a more equitable liver graft allocation (Washburn et al. 2010).

A national conference on liver allocation for HCC patients in the United States recommended that an HCC priority score needs to be developed, which incorporates the calculated MELD score, serum AFP, tumor size, and rate of growth (Pomfret et al. 2010). Toso et al. (2012) proposed such a model, including tumor number in addition to AFP and tumor size (Toso et al. 2012). This new dropout equivalent MELD (DeMELD) score was validated in two patient cohorts in the United States and United Kingdom and found to provide a dynamic and more equitable estimation of dropout risk than the use of exception MELD points (Toso et al. 2014).

Disadvantage of the HCC Dropout Probability Scores

Adopting HCC dropout probability scores will prioritize patients with higher dropout risk for liver transplant. This may have negative impact on posttransplant outcome since those patients have more advanced HCC, with bigger tumor sizes and higher AFP levels (Cucchetti et al. 2011b). In other words, the higher the dropout probability scores, the higher the predicted tumor recurrence and the lower the posttransplant survival. This is simply because factors affecting dropout risk and posttransplant survival are basically the same (Cucchetti et al. 2011b).

Pretransplant Locoregional Treatment (LRT) for HCC and Their Indications

LRT of HCC lesions in potential transplant candidates, either before listing or while being on the transplant waiting list, has become the standard of care in most transplant centers worldwide (Cescon et al. 2013). There are two main indications for LRT in those patients. The first is to stabilize tumors initially meeting transplant selection criteria in order to prevent or reduce their progression beyond the transplantable limits and consequently their dropout of the transplant waiting list. This strategy is called "bridging," and it is most beneficial for patients with T2 tumors with more than 6 months estimated waiting time on the transplant list (Majno et al. 2011). The second indication is to reduce tumor burden exceeding transplant selection criteria in order to meet those criteria and qualify for liver transplantation. This strategy is called "down-staging," and it was initially recommended by the group from L'Hopital Paul Brousse, Paris, France, in 1997 (Galuppo et al. 2013). A period of follow-up is required to verify response to treatment before considering down-staging successful and listing those patients for transplantation (Cescon et al. 2013).

LRTs include transarterial chemoembolization (TACE), transarterial radioembolization (TARE), radio-frequency ablation (RFA), and percutaneous ethanol injection (PEI) in addition to other new modalities (Toso et al. 2010). TACE and percutaneous ablations are the treatments most frequently used in transplant candidates (Cescon et al. 2013; Toso et al. 2010). In addition to reduction of dropout of

listed candidates and down-staging of noncandidates to qualify for transplant, TACE before liver transplant may have other indications including detection of additional nodules missed by CT scan and ultrasound, prediction of posttransplant disease recurrence, and selection of suitable candidates for transplantation (Cucchetti et al. 2011a; Otto et al. 2006, 2013). The effect of LRT depends on how much pathologic tumor necrosis will be achieved (Agopian et al. 2015b). The reported rates of complete pathologic response ranged from 27% to 57% after TACE and from 47% to 75% after thermal ablation (Agopian et al. 2015b; Pompili et al. 2013).

AFP Response to LRT Predicts Treatment Outcome

AFP has been studied as a possible predictor of response to LRT in HCC patients. Riaz et al. (2009) showed that AFP response (defined as more than 50% decrease from a baseline AFP >200 ng/mL) is a reliable predictor of radiologic response, tumor progression, and overall survival for HCC patients treated with LRT. He also suggested that AFP response was able to predict treatment response earlier than imaging (Riaz et al. 2009). The study however didn't include data on liver transplantation.

Agopian et al. (2015b) studied AFP among other predictive factors of a complete pathologic response in 501 patients with HCC treated with LRT before liver transplantation. He reported that median AFP levels (both maximum and immediate pretransplant values) were significantly lower in patients with complete pathologic response and that reduction of AFP after LRT compared to maximum values was an independent predictor of complete pathologic response, which correlated significantly with recurrence-free survival after liver transplantation (Agopian et al. 2015b).

Bridging Effect of LRT and AFP Predictive Value

Whereas older studies showed no specific impact of LRT on waiting list dropout, the most recent series have shown lower dropout rates between 3.0% and 9.3% with bridging strategies (Pompili et al. 2013). Frangakis et al. (2011) reported a significant reduction of waiting list dropout for HCC patients treated with TACE to 3% versus 15% for untreated patients, respectively ($P = 0.04$). The 2-year survival was better for the TACE group, but it did not reach statistical significance (76.0% vs. 57.3% $P = 0.078$) (Frangakis et al. 2011). The current recommendation is that using LRT for bridging HCC patients on the transplant wait list is not harmful and may be beneficial for patients with T2 lesions and an expected waiting time on the transplant list of more than 6 months (Majno et al. 2011; Pomfret et al. 2010).

AFP measured before or after LRT may predict the success of bridging treatment and dropout risk. (Cucchetti et al. 2011a) reported that nodule number and AFP >400 ng/mL at diagnosis of HCC were the tumor-related factors that significantly correlated with response to bridging therapy at 3 months posttreatment (Cucchetti

et al. 2011a). On the other hand, Mehta et al. (2013) found that an AFP >20 ng/mL after the first LRT is a significant predictor of dropout in multivariate analysis and that the level of AFP significantly correlates with the 1-year cumulative incidence of dropout. The study reported significantly higher dropout rate of 59.5% in patients with AFP >500 ng/mL compared to 12.7% in those with AFP ≤20 ng/mL (Mehta et al. 2013).

AFP and Definition of Down-Staging

Down-staging HCC lesions exceeding Milan or UCSF criteria is a valid alternative to the controversial expansion of the standard selection criteria (Pompili et al. 2013). Different LRTs have been used for down-staging of advanced HCC lesions with variable success rates (Gordon-Weeks et al. 2011). A recent systemic review reported successful down-staging in 24–69% of HCC patients with posttransplant recurrence-free and overall survival rates comparable to patients initially within Milan criteria (Gordon-Weeks et al. 2011). However, authors reported not only variable inclusion criteria for down-staging but also different definitions of successful down-staging. A US-National Consensus Conference recommended eligibility criteria for down-staging to include a single tumor ≤8 cm or 2–3 tumors, each ≤5 cm, with a TTD ≤8 cm and no vascular invasion by imaging studies. The conference also defined successful down-staging as a residual tumor meeting Milan criteria in addition to reduction of serum AFP to <500 ng/mL for those patients with an initial AFP >1000 ng/mL (Pomfret et al. 2010).

AFP Predicts Down-Staging Success

High AFP levels before LRT were reported in a number of studies as a significant predictor of down-staging failure. Barakat et al. (2010) reported significantly higher mean AFP levels in the non-down-staged group of HCC patients compared to the down-staged group (5670 ng/mL vs. 799 ng/mL, $P < 0.048$) (Barakat et al. 2010). Yao et al. (2008) reported successful down-staging in 70.5% of a cohort of 61 patients with HCC exceeding Milan criteria and found that pretreatment AFP >1000 ng/mL was the only significant variable predicting down-staging failure (Yao et al. 2008). In a recent study by the same author including a larger cohort of 118 patients with HCC, AFP >1000 ng/mL was again found to be a significant predictor of dropout in the down-staged patient group (Yao et al. 2015).

On the other hand, a low AFP level was reported as an independent predictor of effective LRT. Bova et al. (2013) showed that an AFP level <100 ng/mL predicted successful down-staging of HCC lesions beyond Milan criteria treated with intra-arterial therapy (TACE or TARE) (Bova et al. 2013).

AFP After Down-Staging Can Predict Transplant Outcome

AFP changes after down-staging were shown by a number of studies to predict transplant outcome. As mentioned before, Merani et al. (2011) reviewed data of 6817 HCC patients from the Scientific Registry of Transplant Recipients and found that patients with AFP reduced to ≤400 ng/mL at transplantation, as a result of successful down-staging, had significantly better 3-year intent-to-treat survival than those who failed to reduce their AFP to ≤400 ng/mL (81% vs. 48%, $P \leq 0.001$). Moreover, there was no significant survival difference between patients with AFP reduced to ≤400 ng/mL and those with AFP persistently below 400 ng/mL ($P = 0.14$) (Merani et al. 2011).

As mentioned earlier, the study of Berry et al. (2013) showed the significance of AFP rather than tumor burden predicting survival after liver transplantation. An equally significant conclusion of the study was that down-staging of serum AFP was associated with down-staging of posttransplant mortality. Patients whose AFP decreased (from >320 to ≤320 or from 16–320 to 0–15 ng/mL) had posttransplant mortality similar to the group of patients with the lower AFP range. So, patients initially expected to have poor survival because of high AFP levels can still have better posttransplant outcome with down-staging if their serum AFP levels subsequently decrease (Berry and Ioannou 2013).

Similarly, but with higher AFP figures, Hameed et al. (2014) showed that patients with an initial AFP level >1000 ng/mL that subsequently decreased to <1000 ng/mL after LRT had lower recurrence risk and more favorable prognosis after liver transplantation (Hameed et al. 2014).

Since elevation of AFP is associated with higher posttransplant HCC recurrence risk that may be reduced with pretransplant LRT, AFP monitoring for HCC transplant candidates while on the waiting list has been recommended by a recent consensus conference on HCC management (Clavien et al. 2012).

AFP Monitoring After Liver Transplantation

Patients transplanted for HCC have an 8–20% risk of tumor recurrence, which usually occurs in the first 2 years after transplantation (Zimmerman et al. 2008). Few studies addressed posttransplant monitoring protocols for HCC patients. Even though AFP has no longer been part of the screening and diagnosis of HCC in most of the recent guidelines, yet its monitoring has been suggested for HCC screening after liver transplantation. The recommended protocol involves a contrast-enhanced CT or MRI every 6–12 months and AFP measurement. More frequent imaging hasn't been proven cost-effective, but it is indicated in the presence of abnormal AFP concentrations (Clavien et al. 2012).

Predictive Value of Posttransplant AFP Levels

Apart from screening for HCC recurrence, literature reporting significance of AFP monitoring after transplantation is scarce. A study from Japan highlighted factors predicting survival at the time of HCC recurrence after live donor liver transplantation in 167 recipients with HCC. AFP ≥300 ng/mL at recurrence was a significant predictor of poor prognosis among other factors, the most important of which was neutrophil/lymphocyte ratio (NLR) of ≧4 (Harimoto et al. 2013).

Other Tumor Markers for HCC

Due to the limitations of AFP in diagnosis of HCC, other tumor markers have been used alone or in combination with AFP for diagnosis and prediction of prognosis of HCC

- **AFP-L3**
  Lens culinaris agglutinin-reactive AFP (AFP-L3) is a variant of AFP that is expressed as a percentage of the total AFP. It was reported to have higher sensitivity and specificity for HCC at a cutoff >15%. However, it is of limited usefulness since it was mainly studied in Asian populations who already have elevated AFP (Kim et al. 2016).
- **Des-γ-Carboxyprothrombin (DCP)**
  Prothrombin-induced vitamin K absence-II (PIVKA-II) is an abnormal prothrombin found in the sera of HCC patients. Measured alone, it showed low sensitivity (48–62%) but good specificity for HCC (Kim et al. 2016). Studies combining measurement of both AFP and DCP reported wide ranges of sensitivity (48–94%) and specificity (53–99%), which may be higher than those for either tumor marker alone (Song et al. 2016).

  DCP has been used as a predictor of HCC recurrence after liver resection, LRT, and eventually liver transplantation. Also, it has been incorporated in different liver transplant selection criteria with either tumor morphology (Kyoto group (Fujiki et al. 2009)), AFP (the A-P level (Todo et al. 2007)), or both (Tokyo criteria (Shindoh et al. 2014)).
- **MicroRNAs**
  MicroRNAs are small non-coding RNA molecules that regulate gene expression. MicroRNAs can be released into the circulation and can be detected in body fluids. Circulating miRNAs are associated with HCC and have been studied as possible biomarkers with promising results (Borel et al. 2012). Zhou et al. conducted a study including 934 persons to study microRNA panel (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801). They reported a satisfactory performance of the microRNA panel in diagnosis

of HCC regardless of its stage. MicroRNA panel could differentiate HCC from healthy, chronic hepatitis B, and cirrhosis (Zhou et al. 2011).

– **Other several biomarkers** have been studied and showed promising results but still not recommended for surveillance and diagnosis of HCC. These include glypican-3 (a heparin sulfate proteoglycan), heat shock proteins, vascular endothelial growth factor, interleukin-8, transforming growth factor-beta 1, tumor-specific growth factor, and squamous cell carcinoma antigen (Kim et al. 2016).

Summary Points

- AFP is neither sensitive nor specific for HCC and is no longer used for HCC surveillance or diagnosis as per most of the recent guidelines.
- The recommended surveillance protocol for HCC is by ultrasound examination only every 6 months.
- Diagnosis of HCC is radiological by demonstration of the typical enhancement pattern of HCC on 4-phase dynamic cross-sectional imaging studies with liver mass biopsy reserved for equivocal cases.
- The staging algorithm of the Barcelona Clinic Liver Cancer (BCLC) is the most beneficial and widely used staging system.
- Liver transplantation is the gold standard treatment for selected patients with chronic liver disease and HCC.
- Milan criteria remain the gold standard liver transplant selection criteria for HCC patients.
- Higher pretransplant absolute AFP values >1000 ng/mL correlate with microvascular invasion, poor differentiation, higher posttransplant HCC recurrence, and poor survival.
- Lower AFP cutoff values were suggested by different studies, and there is no universal agreement on a specific cutoff value till now.
- A rising AFP slope of ≥ 15 ng/mL/month may be more significant than static AFP values predicting posttransplant recurrence and poor survival.
- Several transplant selection criteria were proposed incorporating AFP with different tumor parameters as the number and size, total volume, total diameter, etc. In spite of the promising results that compare to Milan, none of these criteria have been universally agreed upon or recommended by the most recent guidelines.
- The AFP model of Duvoux et al. (2012) has been validated and appears to be an accurate prognostic model compared to other criteria including Milan criteria.
- Incorporating pretransplant AFP into Milan criteria may exclude more candidates from liver transplantation, yet it may improve the performance of Milan criteria in prediction of posttransplant HCC recurrence and survival.
- LRTs can be used for "bridging" patients with T2 tumors to liver transplant if the expected waiting time on the list is ≥ 6 months.

- Dropout probability scores incorporating AFP with tumor parameters and MELD score were proposed for prioritization of HCC patients for liver transplant instead of the exception MELD points, but not yet agreed upon.
- Down-staging is a valid alternative to the controversial expansion of transplant selection criteria.
- AFP can predict the outcome of LRTs for bridging/down-staging HCC patients.
- Reduction of AFP as a result of successful down-staging predicts favorable posttransplant outcome.
- It is recommended to monitor AFP pre- and posttransplantation for HCC patients.
- High AFP at the time of diagnosis of HCC recurrence after transplantation may predict treatment failure and poor prognosis.
- Other biological markers as AFP-L3 and DCP may have a role in diagnosis of HCC.
- A number of new biomarkers and genetic markers related to HCC are being studied with promising results.

References

Adachi Y, Tsuchihashi J, Shiraishi N, Yasuda K, Etoh T, Kitano S. AFP-producing gastric carcinoma: multivariate analysis of prognostic factors in 270 patients. Oncology. 2003; 65(2):95–101.

Adinolfi A, Adinolfi M, Lessof. Alpha-feto-protein during development and in disease. J Med Genet. 1975;12(2):138–51. Pubmed Central PMCID: 1013256.

Agopian VG, Harlander-Locke M, Zarrinpar A, Kaldas FM, Farmer DG, Yersiz H, et al. A novel prognostic nomogram accurately predicts hepatocellular carcinoma recurrence after liver transplantation: analysis of 865 consecutive liver transplant recipients. J Am Coll Surg. 2015a; 220(4):416–27.

Agopian VG, Morshedi MM, McWilliams J, Harlander-Locke MP, Markovic D, Zarrinpar A, et al. Complete pathologic response to pretransplant locoregional therapy for hepatocellular carcinoma defines cancer cure after liver transplantation: analysis of 501 consecutively treated patients. Ann Surg. 2015b;262(3):536–45. discussion 43–5.

Barakat O, Wood RP, Ozaki CF, Ankoma-Sey V, Galati J, Skolkin M, et al. Morphological features of advanced hepatocellular carcinoma as a predictor of downstaging and liver transplantation: an intention-to-treat analysis. Liver Transpl. 2010;16(3):289–99.

Benson 3rd AB, D'Angelica MI, Abrams TA, Are C, Bloomston PM, Chang DT, et al. Hepatobiliary cancers, version 2.2014. J Natl Compr Canc Netw. 2014;12(8):1152–82.

Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society. 2013 Jun;19(6):634-45. PubMed PMID: 23536495.

Bismuth H, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. Ann Surg. 1993; 218(2):145–51. Pubmed Central PMCID: 1242923, Epub 1993/08/01. eng.

Borel F, Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol. 2012;56(6):1371–83.

Bova V, Miraglia R, Maruzzelli L, Vizzini GB, Luca A. Predictive factors of downstaging of hepatocellular carcinoma beyond the Milan criteria treated with intra-arterial therapies. Cardiovasc Intervent Radiol. 2013;36(2):433–9.

Bruix J, Sherman M, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2. Pubmed Central PMCID: 3084991.

Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V. Liver cancer: approaching a personalized care. J Hepatol. 2015;62(1 Suppl):S144–56. Pubmed Central PMCID: 4520430.

Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology. 2016;150(4):835–53.

Busuttil RW. Liver transplantation for hepatocellular carcinoma: the Hangzhou experience. Hepatobiliary Pancreat Dis Int. 2008;7(3):235–6.

Cescon M, Cucchetti A, Ravaioli M, Pinna AD. Hepatocellular carcinoma locoregional therapies for patients in the waiting list. Impact on transplantability and recurrence rate. J Hepatol. 2013;58(3):609–18.

Chaiteerakij R, Zhang X, Addissie BD, Mohamed EA, Harmsen WS, Theobald PJ, et al. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. Liver Transpl. 2015;21(5):599–606. Pubmed Central PMCID: 4490162.

Clavien P-A, Lesurtel M, Bossuyt PMM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. Lancet Oncol. 2012;13(1):e11–22.

Cucchetti A, Cescon M, Bigonzi E, Piscaglia F, Golfieri R, Ercolani G, et al. Priority of candidates with hepatocellular carcinoma awaiting liver transplantation can be reduced after successful bridge therapy. Liver Transpl. 2011a;17(11):1344–54.

Cucchetti A, Cescon M, Bertuzzo V, Bigonzi E, Ercolani G, Morelli MC, et al. Can the dropout risk of candidates with hepatocellular carcinoma predict survival after liver transplantation? Am J Transplant. 2011b;11(8):1696–704.

Deutsch HF. Chemistry and biology of alpha-fetoprotein. Adv Cancer Res. 1991;56:253–312.

DuBay DA, Sandroussi C, Kachura JR, Ho CS, Beecroft JR, Vollmer CM, et al. Radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. HPB. 2011; 13(1):24–32. Pubmed Central PMCID: 3019538.

Duffy JP, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, et al. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. Ann Surg. 2007;246(3):502–9. Pubmed Central PMCID: 1959350, discussion 9–11.

Dumitra TC, Dumitra S, Metrakos PP, Barkun JS, Chaudhury P, Deschenes M, et al. Pretransplantation alpha-fetoprotein slope and Milan criteria: strong predictors of hepatocellular carcinoma recurrence after transplantation. Transplantation. 2013;95(1):228–33.

Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al. Liver transplantation for hepatocellular carcinoma: a model including alpha-fetoprotein improves the performance of Milan criteria. Gastroenterology. 2012;143(4):986–94.e3; quiz e14-5.

El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatology. 2014;60(5):1767–75. Pubmed Central PMCID: 4211957.

European Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43.

Frangakis C, Geschwind JF, Kim D, Chen Y, Koteish A, Hong K, et al. Chemoembolization decreases drop-off risk of hepatocellular carcinoma patients on the liver transplant list. Cardiovasc Intervent Radiol. 2011;34(6):1254–61. Pubmed Central PMCID: 4137764.

Freeman RB, Edwards EB, Harper AM. Waiting list removal rates among patients with chronic and malignant liver diseases. Am J Transplant. 2006;6(6):1416–21.

Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, et al. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. Am J Transplant. 2009;9(10):2362–71.

Galuppo R, McCall A, Gedaly R. The role of bridging therapy in hepatocellular carcinoma. Int J Hepatol. 2013;2013:419302. Pubmed Central PMCID: 3880689.

Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. Hepatology. 2002;36(5 Suppl 1):S84–92.

Gitlin D, Perricelli A, Gitlin GM. Synthesis of -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res. 1972;32(5):979–82.

Golfieri R, Coppola F, Fusco F, Li Bassi S, Caraceni P, Bernardi M, et al. Malignant progression of a small HCC nodule: hypovascular "early HCC" converted to hypervascular "small HCC" within six months. Dig Liver Dis. 2007;39(9):883–90.

Gordon-Weeks AN, Snaith A, Petrinic T, Friend PJ, Burls A, Silva MA. Systematic review of outcome of downstaging hepatocellular cancer before liver transplantation in patients outside the Milan criteria. Br J Surg. 2011;98(9):1201–8.

Gouw AS, Balabaud C, Kusano H, Todo S, Ichida T, Kojiro M. Markers for microvascular invasion in hepatocellular carcinoma: where do we stand? Liver Transpl. 2011;17 Suppl 2:S72–80.

Hakeem AR, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. Aliment Pharmacol Ther. 2012;35(9):987–99.

Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level $>$ 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. Liver Transpl. 2014;20(8):945–51. Pubmed Central PMCID: 4807739.

Han K, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, et al. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. J Can Gastroenterol. 2007;21(1):39–45. Pubmed Central PMCID: 2656629.

Harimoto N, Shirabe K, Nakagawara H, Toshima T, Yamashita Y, Ikegami T, et al. Prognostic factors affecting survival at recurrence of hepatocellular carcinoma after living-donor liver transplantation: with special reference to neutrophil/lymphocyte ratio. Transplantation. 2013;96(11):1008–12.

Harper ME, Dugaiczyk A. Linkage of the evolutionarily-related serum albumin and alpha-fetoprotein genes within q11-22 of human chromosome 4. Am J Hum Genet. 1983;35 (4):565–72. Pubmed Central PMCID: 1685723.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.

Jonas S, Bechstein WO, Steinmuller T, Herrmann M, Radke C, Berg T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology. 2001;33(5):1080–6.

Kashkoush S, El Moghazy W, Kawahara T, Gala-Lopez B, Toso C, Kneteman NM. Three-dimensional tumor volume and serum alpha-fetoprotein are predictors of hepatocellular carcinoma recurrence after liver transplantation: refined selection criteria. Clin Transplant. 2014;28(6):728–36.

Kim JU, Shariff MI, Crossey MM, Gomez-Romero M, Holmes E, Cox IJ, et al. Hepatocellular carcinoma: review of disease and tumor biomarkers. World J Hepatol. 2016;8(10):471–84. Pubmed Central PMCID: 4820639.

Lai Q, Melandro F, Pinheiro RS, Donfrancesco A, Fadel BA, Levi Sandri GB, et al. Alpha-fetoprotein and novel tumor biomarkers as predictors of hepatocellular carcinoma recurrence after surgery: a brilliant star raises again. Int J Hepatol. 2012a;2012:893103. Pubmed Central PMCID: 3391901.

Lai Q, Avolio AW, Manzia TM, Sorge R, Agnes S, Tisone G, et al. Combination of biological and morphological parameters for the selection of patients with hepatocellular carcinoma waiting for liver transplantation. Clin Transplant. 2012b;26(2):E125–31.

Lai Q, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, et al. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. Liver Transpl. 2013;19 (10):1108–18.

Lai Q, Inostroza M, Rico Juri JM, Goffette P, Lerut J. Delta-slope of alpha-fetoprotein improves the ability to select liver transplant patients with hepatocellular cancer. HPB. 2015;17(12):1085–95. Pubmed Central PMCID: 4644360.

Lao OB, Weissman J, Perkins JD. Pre-transplant therapy for hepatocellular carcinoma is associated with a lower recurrence after liver transplantation. Clin Transplant. 2009;23(6):874–81.

Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. Hepatology. 1999;30(6):1434–40.

Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. Gastroenterology. 2010;138(2):493–502. Pubmed Central PMCID: 2819612.

Mailey B, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, et al. Evaluation of absolute serum alpha-fetoprotein levels in liver transplant for hepatocellular cancer. Arch Surg. 2011;146 (1):26–33.

Majno P, Lencioni R, Mornex F, Girard N, Poon RT, Cherqui D. Is the treatment of hepatocellular carcinoma on the waiting list necessary? Liver Transpl. 2011;17 Suppl 2:S98–108.

Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334(11):693–9. Epub 1996/03/14. eng.

Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol. 2009;10(1):35–43. Epub 2008/12/09. eng.

Mazzaferro V, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, et al. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. Liver Transpl. 2011;17 Suppl 2:S44–57. Epub 2011/06/23. eng.

Mehta N, Dodge JL, Goel A, Roberts JP, Hirose R, Yao FY. Identification of liver transplant candidates with hepatocellular carcinoma and a very low dropout risk: implications for the current organ allocation policy. Liver Transpl. 2013;19(12):1343–53. Pubmed Central PMCID: 3883622.

Merani S, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, et al. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. J Hepatol. 2011;55(4):814–9.

Moya A, Berenguer M, Aguilera V, Juan FS, Nicolas D, Pastor M, et al. Hepatocellular carcinoma: can it be considered a controversial indication for liver transplantation in centers with high rates of hepatitis C? Liver Transpl. 2002;8(11):1020–7.

Nikolic JA. Synthesis, structure and function of alpha-fetoproteins and their importance in medicine. Glas Srp Akad Nauka Med. 1992;42:57–73. Sinteza, struktura i funkcija alfa-fetoproteina i njegov znacaj u medicini.

Otto G, Herber S, Heise M, Lohse AW, Monch C, Bittinger F, et al. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. Liver Transpl. 2006;12(8):1260–7.

Otto G, Schuchmann M, Hoppe-Lotichius M, Heise M, Weinmann A, Hansen T, et al. How to decide about liver transplantation in patients with hepatocellular carcinoma: size and number of lesions or response to TACE? J Hepatol. 2013;59(2):279–84.

Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol. 2006;45(4):529–38.

Pomfret EA, Washburn K, Wald C, Nalesnik MA, Douglas D, Russo M, et al. Report of a national conference on liver allocation in patients with hepatocellular carcinoma in the United States. Liver Transpl. 2010;16(3):262–78.

Pompili M, Francica G, Ponziani FR, Iezzi R, Avolio AW. Bridging and downstaging treatments for hepatocellular carcinoma in patients on the waiting list for liver transplantation. World J Gastroenterol. 2013;19(43):7515–30. Pubmed Central PMCID: 3837250.

Riaz A, Ryu RK, Kulik LM, Mulcahy MF, Lewandowski RJ, Minocha J, et al. Alpha-fetoprotein response after locoregional therapy for hepatocellular carcinoma: oncologic marker of radiologic response, progression, and survival. J Clin Oncol. 2009;27(34):5734–42.

Sato Y, Sekine T, Ohwada S. Alpha-fetoprotein-producing rectal cancer: calculated tumor marker doubling time. J Surg Oncol. 1994;55(4):265–8.

Shetty K, Timmins K, Brensinger C, Furth EE, Rattan S, Sun W, et al. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. Liver Transpl. 2004;10(7):911–8.

Shindoh J, Sugawara Y, Nagata R, Kaneko J, Tamura S, Aoki T, et al. Evaluation methods for pretransplant oncologic markers and their prognostic impacts in patient undergoing living donor liver transplantation for hepatocellular carcinoma. Transpl Int. 2014;27(4):391–8.

Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30(1):37–47.

Song P, Feng X, Inagaki Y, Song T, Zhang K, Wang Z, et al. Clinical utility of simultaneous measurement of alpha-fetoprotein and des-γ-carboxy prothrombin for diagnosis of patients with hepatocellular carcinoma in China: a multi-center case-controlled study of 1,153 subjects. BioSci Trends. 2014;8(5):266–73.

Song PP, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, Kokudo N, et al. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. World J Gastroenterol. 2016;22(1):262–74. Pubmed Central PMCID: 4698491.

Sterling RK, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, et al. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. Am J Gastroenterol. 2012;107(1):64–74. Pubmed Central PMCID: 3903319.

Takamori R, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? Liver Transpl. 2000;6(1):67–72.

Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. Hepatol Int. 2008;2(1):17–30. Pubmed Central PMCID: 2716871.

Todo S, Furukawa H, Tada M, Japanese Liver Transplantation Study Group. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. Liver Transpl. 2007;13 (11 Suppl 2):S48–54.

Toso C, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. Hepatology. 2009;49(3):832–8.

Toso C, Mentha G, Kneteman NM, Majno P. The place of downstaging for hepatocellular carcinoma. J Hepatol. 2010;52(6):930–6.

Toso C, Dupuis-Lozeron E, Majno P, Berney T, Kneteman NM, Perneger T, et al. A model for dropout assessment of candidates with or without hepatocellular carcinoma on a common liver transplant waiting list. Hepatology. 2012;56(1):149–56.

Toso C, Majno P, Berney T, Morel P, Mentha G, Combescure C. Validation of a dropout assessment model of candidates with/without hepatocellular carcinoma on a common liver transplant waiting list. Transpl Int. 2014;27(7):686–95.

Varona MA, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, Diaz D, et al. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. Transplant Proc. 2015;47(1):84–9.

Vibert E, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, et al. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. Am J Transplant. 2010;10(1):129–37.

Washburn K, Edwards E, Harper A, Freeman R. Hepatocellular carcinoma patients are advantaged in the current liver transplant allocation system. Am J Transplant. 2010;10(7):1643–8.

Xu X, Lu D, Ling Q, Wei X, Wu J, Zhou L, et al. Liver transplantation for hepatocellular carcinoma beyond the Milan criteria. Gut 2015;0: 1–7.

Yamashiki N, Gaynor JJ, Kato T, Reddy KR, Sobhonslidsuk A, Levi D, et al. Competing risks analysis of predictors of delisting owing to tumor progression in liver transplant candidates with hepatocellular carcinoma. Am J Transplant. 2004;4(5):774–81.

Yang SH, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, et al. A revised scoring system utilizing serum alphafetoprotein levels to expand candidates for living donor transplantation in hepatocellular carcinoma. Surgery. 2007;141(5):598–609.

Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology. 2001;33(6):1394–403.

Yao FY, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. Am J Transplant. 2007;7(11):2587–96.

Yao FY, Kerlan Jr RK, Hirose R, Davern 3rd TJ, Bass NM, Feng S, et al. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. Hepatology. 2008;48(3):819–27. Pubmed Central PMCID: 4142499.

Yao FY, Mehta N, Flemming J, Dodge J, Hameed B, Fix O, et al. Downstaging of hepatocellular cancer before liver transplant: long-term outcome compared to tumors within Milan criteria. Hepatology. 2015;61(6):1968–77. Pubmed Central PMCID: 4809192.

Zheng SS, Xu X, Wu J, Chen J, Wang WL, Zhang M, et al. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. Transplantation. 2008;85(12):1726–32.

Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol. 2011;29(36):4781–8.

Zimmerman MA, Ghobrial RM, Tong MJ, Hiatt JR, Cameron AM, Hong J, et al. Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. Arch Surg. 2008;143(2):182–8. discussion 8.

Zou WL, Zang YJ, Chen XG, Shen ZY. Risk factors for fatal recurrence of hepatocellular carcinoma and their role in selecting candidates for liver transplantation. Hepatobiliary Pancreat Dis Int. 2008;7(2):145–51.