**Name of journal:** **World Journal of** **Gastroenterology**

**ESPS Manuscript NO: 19234**

**Manuscript Type: TOPIC HIGHLIGHTS**

2015 Advances in Hepatocellular Carcinoma

**Prediction of hepatocellular carcinoma biological behavior in patient selection for liver transplantation**

Umberto C *et al.* Evaluation of HCC aggressiveness for LT

Cillo Umberto, Giuliani Tommaso, Polacco Marina, Herrero Manley Luz Maria, Crivellari Gino, Vitale Alessandro

**Cillo Umberto, Giuliani Tommaso, Polacco Marina, Herrero Manley Luz Maria, Vitale Alessandro**, Hepatobiliary Surgery and Liver Transplantation Unit, Department of General Surgery and Organ Transplantation, University Hospital of Padua, 35128 Padua, Italy

**Crivellari Gino,** Unità Operativa di Oncologia Medica, Istituto Oncologico Veneto (IOV) IRCCS, 35128 Padova, Italy

**Author contributions:** Cillo U designed the research; Giuliani T and Polacco M performed the research; Cillo U, Giuliani T, Polacco M, Herrero Manley L wrote the paper; Cillo U, Giuliani T, Polacco M, Herrero Manley L, Crivellari G and Vitale A revised the paper.

**Conflict-of-interest** **statement:** No potential conflicts of interest relevant to this article were reported.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to:** **Umberto Cillo, MD, FEBS,** Hepatobiliary Surgery and Liver Transplantation Unit, Department of General Surgery and Organ Transplantation, University Hospital of Padua, Via 8 Febbraio, 35128 Padova, Italy. cillo@unipd.it

**Telephone**: +39-49-8211846

**Fax**: +39-49-8211718

**Received:** April 30, 2015

**Peer-review started:** May 8, 2015

**First decision:** July 14, 2015

**Revised:** August 14, 2015

**Accepted:** November 9, 2015

**Article in press:**

**Published online:**

**Abstract**

Morphological criteria have always been considered the benchmark for selecting hepatocellular carcinoma (HCC) patients for liver transplantation (LT). These criteria, which are often inappropriate to express the tumor’s biological behavior and aggressiveness, offer only a static view of the disease burden and are frequently unable to correctly stratify the tumor recurrence risk after LT. Alpha-fetoprotein (AFP) and its progression as well as AFP-mRNA, AFP-L3%, des-γ-carboxyprothrombin, inflammatory markers and other serological tests appear to be correlated with post-transplant outcomes. Several other markers for patient selection including functional imaging studies such as 18F-FDG-PET imaging, histological evaluation of tumor grade, tissue-specific biomarkers, and molecular signatures have been outlined in the literature. HCC growth rate and response to pre-transplant therapies can further contribute to the transplant evaluation process of HCC patients. While AFP, its progression, and HCC response to pre-transplant therapy have already been used as a part of an integrated prognostic model for selecting patients, the utility of other markers in the transplant setting is still under investigation. This article intends to review the data in the literature concerning predictors that could be included in an integrated LT selection model and to evaluate the importance of biological aggressiveness in the evaluation process of these patients.

**Key words:** Hepatocellular carcinoma; Liver Transplantation; Biomarkers; Alpha-fetoprotein; Histopathology; Recurrence; integrated prognostic tool

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** An integrated model predicting post-transplant survival of hepatocellular carcinoma patients after liver transplantation has not yet been defined. Current selection criteria for liver transplantation that do not consider its biological aggressiveness are mainly based on morphological tumor markers that offer only a static view of the tumor. Many biomarkers predicting post-transplant outcome and stratifying those patients who are candidates for liver transplantation are under evaluation. An integrated prognostic model will make it possible to quantify the tumor burden via functional imaging modalities as well as biological markers.

Umberto C, Tommaso G, Marina P, Manley H, Maria ML, Gino C, Alessandro V. Prediction of hepatocellular carcinoma biological behavior in patient selection for liver transplantation.*World J Gastroenterol* 2015; In press

**INTRODUCTION**

Liver transplantation is the gold standard treatment for selected patients with hepatocellular carcinoma (HCC) as it cures both the tumor and the underlying liver cirrhosis. Since widespread use of liver transplantation (LT) is still limited due to organ shortage[1], reliable patient selection criteria are critical to maximize LT survival benefit. The equipoise between the impact that decision will have on the patients remaining on the waiting list and on the recipient him/herself must be based on reliable predictors of post-transplant outcome.

Explant pathology features constitute a direct expression of the tumor’s biological aggressiveness and, in particular, of micromacrovascular invasion and dedifferentiated grading. The former, in particular, represents the most important marker of HCC aggressiveness[2-6], Iwatsuki *et al*[7] demonstrated a more than 4-fold increased risk of recurrence following transplant when microvascular invasion is detecte. Although associated with an excellent ability to predict post-transplant HCC recurrence, microvascular invasion is difficult to detect at the pre-operative biopsy. Crucial information needed to predict the outcome and to guide the decision-making process about listings becomes available, therefore, only after the explant specimen has undergone pathologic examination. Although research has been attempting to identify new makers, gross morphology has until now been considered the primary prognostic parameter. Strict adherence to macromorphological criteria (*i.e.,* the Milan criteria) has been considered the best selection criteria for HCC patients in the transplant setting since the sizes and the number of the nodules are considered the best surrogates for microvascular invasion. Indeed, nodule size and number are the worldwide standard for patient selection in most centers. The Milan, the University of California, San Francisco (UCSF), the total tumor volume (TTV) and the Up-to-7, which[8,9] are associated with good overall middle to long term disease-free survival (DFS) rates after transplantation[10,11]*,* are the most frequently used and scientifically validated morphology-based criteria.

It is widely recognized, nevertheless, that use of pre-transplant macromorphological criteria in the selection process of LT candidates poses a number of relevant drawbacks. It has likewise been shown that in a large proportion of patients (up to 15%-25%)[12]. There is a significant discrepancy between pre-transplant radiologic staging and explant pathology. These findings are partially attributable to the time-lapse between the last radiologic evaluation and the transplant itself during which the tumor and the staging may have progressed. The discrepancy may also be explained by inaccurate radiological data leading to an underestimation of nodule number/size. There could also be an incomplete overlapping of macromorphological traits and absence of microvascular invasion. In fact, although infrequent, small paucinodular HCC may present biologically aggressive features that would seem to predict an unfavorable post-transplant outcome. Finally, and more importantly, the strict adoption of macromorphologicical criteria could lead to the exclusion of a relevant number of patients who could, instead, benefit from transplant[13-17]. Despite being outside current transplant criteria, some multinodular HCCs and also, to a lesser extent, relatively large tumors, have been shown to possess a favorable biological behavior and an acceptable long term post-transplant DFS. In view of these findings, several clinical trials have focused on expanding the Milan criteria[9,18]. According to “the metroticket concept”[8], nevertheless, the further HCC staging criteria is expanded for LT, the greater the cost will be in terms of higher recurrence. Conversely, when morphological selection protocols such as the Milan one include other recurrence predictors, their performance in predicting tumor recurrence seems to be improved[19,20] , thus suggesting that other prognostic factors do exist and may be useful in improving prognostic accuracy. These considerations underline how a better understanding of the proliferative activity of HCC tumors can help to improve the selection criteria for LT and optimize resource allocation.

Although a definitive characterization is at yet unattainable, much light has recently been shed on the assessment of HCC tumor aggressiveness; these data and our own findings are the object of the present article, which has been divided into five sections: (1) biohumoral markers; (2) radiologic features; (3) histology; (4) response to therapy; and (5) tumor doubling times.

**BIOHUMORAL MARKERS**

***AFP***

First described by Abelev *et al*[21] in 1963, alpha-fetoprotein (AFP) is a usually fetal-specific glycoprotein whose importance in the diagnosis of HCC is well established[22-25]. Increasing evidence[8,11,26-29] suggests, moreover, that it also has a role in predicting outcome after LT. Since many studies[30-33] have reported a correlation between pre-LT serum AFP and post-LT overall survival in patients with HCC, AFP is considered an independent predictor of post-transplant survival (Table 1). Todo *et al*[30] reported 1, 3, and 5-year survival rates, respectively, of 84%, 77%, 72% for AFP < 200 μg/L *vs* 65%, 42%, 34% for AFP > 1000 μg/L. Mailey *et al*[32] likewise showed that the 1, 3, and 5-year absolute survival rates of 92%, 82%, 74%, respectively, in patients whose AFP level was lower than 20 μg/L decreased to 82%, 63%, 52% among those with an AFP > 400 μg/L.

Berry *et al*[31] reported that transplant recipients with HCC and serum AFP levels ≤ 15 ng/mL at transplant did not have a higher post-transplant mortality (AHR = 1.03) with respect to those without HCC. Patients with 16 to 65 ng/mL (AHR = 1.38), 66 -320 ng/mL (AHR = 1.65), and > 320 ng/mL (AHR = 2.37) serum AFP levels had progressively worse post-transplant mortality rates in comparison with recipients without HCC. Those investigators also reported that patients outside the Milan criteria had excellent outcomes if their AFP levels were < 15 ng/mL, while those who fulfilled the Milan criteria but had high AFP serum levels had poor survival rates.

Other investigators have reported that HCC recurrence after LT was correlated to pre-transplant AFP levels[30,34-41]. Fujiki *et al*[41] demonstrated that the 1, 3, and 5-year recurrence-free survival (RFS) rates in 144 patients was, respectively, 97%, 91%, 90% when AFP was 200 μg/L. However when the AFP was higher than 800 μg/L the RFS were 65%, 40%, 40%. Evaluating 100 HCC transplant patients, Sotiropolous *et al*[37] found 1, 3, and 5-year RFS rates were 100%, 97% and 97% for AFP < 20 μg/L versus 68%, 23% and 23%, respectively, for AFP values higher than 100 μg/L.

Biological behavior features such as vascular invasion and tumor grade[2-7,42] have also been shown to be correlated with AFP levels. Fujiki *et al*[41] demonstrated that AFP > 800 μg/L was associated with an increased risk of microvascular invasion and poor differentiation of HCC with respect to AFP < 200 μg/L. In addition, vascular invasion and tumor differentiation had the highest odds ratios (OS) with AFP levels in a multivariate analysis by Duvoux *et al*[20] .

In view of this evidence, new transplant selection criteria that include AFP have been investigated. Carrying out a study on a population of 6478 patients, Toso *et al*[26] reported that both total TTV and AFP levels were significant predictors of survival. A combined patient selection score based on TTV and AFP was thus developed. Compared to all the other criteria systems tested, that score was found to be the best predictor of outcome (Table 3). Duvoux *et al*[20] subsequently studied a 2 cohort population (with training and validation groups) of patients who had undergone LT for HCC within the context of a multicentric retrospective study and identified 3 independent pre-LT predictors of recurrence: the number of tumors, the tumor size, and the AFP level. These parameters were incorporated to develop a model stratifying low and high risk of recurrence. A 3-tier AFP level score was included in the model and cut-offs of 100 ug/L and 1000 ug/L were adopted to identify the 3 AFP groups. The model proved to have an impact on recurrence and on survival, and net reclassification improvement showed that its predictability was superior to the Milan criteria[20] (Table 3). Duvoux’s AFP model is, in fact, currently used in France (www.agence-biomedecine.fr) and United Kingdom (www.odt.nhs.uk/pdf/advisory\_group\_papers/LAG/HCC\_recommendations\_IR\_TS\_b\_NAS\_Work \_in\_Progress.pdf 19); a value ≤ 2 is used as inclusion criteria for LT in HCC patients. The model has recently received an external validation in Spain[43] as well as in Italy[44], and a recent United States study confirmed the strong prognostic power of an AFP > 1000 ng/mL threshold in HCC patients undergoing LT meeting the Milan Criteria[45].

Although static, and despite the fact that a clear, unanimous cut-off level has yet to be defined, it has been seen AFP does indeed predict outcomes of HCC patients undergoing LT. It is important to remember, however, that a single assessment of serum AFP levels are unable express dynamic changes in the tumor’s biological behavior. Since tumor aggressiveness shows a tendency to progress, at least two time-spaced evaluations are needed to determine if the biological course is stationary or progressing. A study by Kondili *et al*[46] published in 2007 showed that a rapid increase in AFP levels before LT represents a risk factor for tumor recurrence. Another study by Han *et al*[28] demonstrated that AFP progression exceeding 50 μg/L per month was significantly correlated to both vascular invasion and poorly differentiated tumor grade. Vibert *et al*[29] who measured AFP levels once a month in 153 patients on waiting lists for LT with the intent of demonstrating the relevance of dynamic AFP variations, found that its progression was more predictive of tumor recurrence and poor survival after LT than any static value. A slope > 15 μg/L per month was identified as the cut-off value. As suggested by Merani *et al*[47] decreasing AFP values also seem to have a clinical significance. Studying 6817 HCC cases, they reported that patients successfully downstaged from AFP > 400 μg/L to AFP ≤ 400 μg/L had better post-transplant outcomes than patients whose AFP remained > 400 μg/L after downstaging. In addition, both increasing and decreasing AFP levels were found to be relevant to the evaluation of the oncological behavior of HCC, identifying tumors tending toward either a positive or a negative evolution. In view of the biomarker’s potential relevance, the investigators concluded that further studies are warranted to standardize the cut-off values and assessment time points.

Despite abundant data on AFP found in the literature, any conclusions for the time being can only be tentative in view of many unsolved issues. Firstly, since AFP is a biomarker, biases linked to different laboratory methods and processing techniques are unavoidable and comparisons of results from multiple laboratories/studies are uncertain. Secondly, it is probable that the frequent exclusion of LT patients who die within 30 post-operative days has restricted data regarding the most aggressive tumors. According to Hakeem *et al*[48], moreover, patients included in AFP studies are highly heterogeneous. Finally, prognostic evaluations based on AFP levels are made only with regard to patients whose serum levels are higher than normal (> 20 μg/L)[49] despite the fact that a considerable percentage of HCC patients are AFP-negative. In a study by Yang *et al*[50] focusing on novel prognostic biomarkers for HCC, 48.3% of the 305 patients studied had AFP < 20 μg/L. Zhang *et al*[51] likewise reported that 30%–40% of HCC patients studied were AFP-negative. Although a dynamic evaluation of the biomarker (AFP slope) could partially obviate this problem, further studies specifically addressing HCC patients with in-range AFP values are warranted.

***Des-ɤ-carboxyprothrombin***

Des-gamma carboxyprothrombin (DCP), also known as protein induced by vitamin K absence or antagonist II (PIVKA-II), was described more than twenty years after the first description of AFP[52]. The role of DCP as a biomarker for the diagnosis of HCC has been confirmed over the year[53-56], just as has been its potential to detect HCC early, given the highly sensitive immune assay that has been developed[57-59]. DCP’s sensitivity and specificity in diagnosing HCC appear to be better than those of AFP[60-62], and simultaneous testing of both markers has been proposed for tumor detection[63].

Interestingly, DCP has also been shown to be predictive of outcomes regardless of treatment[62,64-67]. Encouraging predictive values were first reported after ablative therapies for HCC[64,65,68]. According to Imamura *et al*[66] DCP was able to predict recurrences after resection for small HCCs and, similarly, Sakaguchi *et al*[67] demonstrated that DCP > 100 mAU/mL was associated to a negative prognosis in HCC patients within the Milan criteria undergoing resection. Some studies have shown that DCP has a predictive significance also with regard to LT outcome and that it is a powerful predictive serum marker. Basing their data on a cohort of 124 patients undergoing living donor liver transplantation (LDLT), Shindoh *et al*[69] found that the prognosis of these patients strongly depended on maximum pre-LT AFP or DCP values. Multivariate analysis performed on 144 HCC patients who underwent LDLT at the Kyoto University showed that DCP > 400 mAU/mL was an independent risk factor for tumor recurrence after transplant.

Fujiki *et al*[70] subsequently published the Kyoto expanded criteria for LDLT which included preoperative DCP levels < 400 mAU/mL, tumor size, and number[41]. A similar proposal was made by Taketomi *et al*[70] who suggested a different cut-off value for DCP (DCP < 300 mAU/mL). The role of DCP was recently confirmed in a United States population. A serum DCP ≥ 7.5 mAU/mL in 127 HCC patients undergoing LT significantly correlated with tumor recurrence (HR = 3.5; 1.9-6.7). The HR increased when DCP was combined with AFP and the Milan criteria. In addition, finding DCP expression in the liver of HCC patients, especially in the peritumoral tissue, both Tang *et al*[74] and Inagaki *et al*[72] suggested that a combination of serum and tissue DCP expression be utilized.

DCP’s prognostic role seems to be linked to its association with elevated cellular proliferation and tumor growth rates[64,75] as well as high infiltrative growth and vascular invasion values[62,72,73,76]. Recently, Potè et al reported that a serum level of DCP > 90 mAU/mL was an independent predictor of vascular invasion, while high DCP tissue expression was associated with poor tumor differentiation[62]. In vitro studies have proven that PIVKA-II is able to promote cellular proliferation and migration[77,78] just as it induces expression of angiogenetic factors such as endothelial grow factor receptor (EGFR) and vascular endothelial grow factor (VEGF)[79,80].

While both clinical and biological reports and in vitro studies support the view that DCP is an index of HCC aggressiveness, its relative clinical relevance is still under debate.

***AFP mRNA***

Post-transplant tumor recurrences are almost certainly due to residual cancer cells from the removed tumor, and detection of HCC cells in the peripheral blood seems to be a direct and accurate method to predict tumor recurrence[81]. At the same time, AFP mRNA expression in the peripheral blood is a reliable marker of circulating cancer cells[82] .

In 2005 our research group reported that the pre-operative AFP mRNA level is a significant predictor of survival after radical therapy for HCC[83]. Marubashi *et al*[84] likewise reported that a positive pre-operative test for peripheral blood AFP mRNA was found to be an independent risk factor for the recurrence of HCC after LDLT.

Using a nested-polymerase chain reaction (PCR) technique, Ljich *et al*[85] reported, instead, that the pre-operative presence of AFP mRNA-expressing cells in the peripheral blood was not associated with after resection HCC recurrence.

Data supporting AFP mRNA’s role as a predictor of HCC recurrence are as yet inconclusive. Toso *et al*[86] hypothesized that strategies to decrease the engrafment of circulating tumor cells could lower the risk of recurrence. Some of the strategies they proposed were selecting recipients with low baseline circulating HCC cells by adding biological markers to the accepted combination of morphological criteria and decreasing the perioperative release of HCC cells via careful perioperative handling of the tumor.

***AFP-L3%***

Given its high specificity and sensitivity in detecting tumors, in the early 90 s some investigators began to consider lens culinaris agglutinin-reactive fraction of α-fetoprotein (AFP-L3%), the percentage of a fucosylated form of AFP over the total AFP level, an adjunctive marker for HCC diagnosis of[87-90].

The biomarker also seems to be able to predict prognosis. High AFP-L3% levels have, in fact, been reported to be correlated with poor outcome after Transcatheter Arterial Chemoembolization (TACE)[91] and with a higher risk of recurrence after local ablation[92,93] and hepatectomy[92,94]. According to Kobayashi *et al*[95] AFP-L3% values are useful in predicting the outcome of patients with normal serum AFP levels[96]. Considering it a potential new generation tumor marker, Kusaba demonstrated that liver cancer cells expressing AFP-L3% showed a tendency towards early vascular invasion and intra-hepatic metastasis, staining more positive with Ki67and less with alpha-catenin[97]. Chaiteerakij *et al*[71] found that AFP-L3% was significantly associated with tumor recurrence in a population of 127 HCC patients undergoing transplantation. Interestingly, the HR increased from 2.6 (1.2-6.6) to 4.5 (1.9-10.6) when that parameter was added to the Milan criteria. A prognostic value was also attributed by that same study to the absolute AFP-L3% value.

There is still little evidence, nevertheless, to support the prognostic relevance of AFP-L3% in the LT context.

**Other biomarkers:** Many other HCC biomarkers after liver resection, loco-regional treatments or LT have been cited in medical literature but their potential prognostic role in the transplant population has yet to be well defined.

Furthermore, the systematic citation of each individual marker is beyond the scope of this review, which focuses on the most accessible and reproducible markers used in daily clinical practice. Nevertheless a brief mention is made for potential subsequent studies into their prognostic value in patients with HCC undergoing LT.

However, one particular biomarker worthy of mention is glypican-3 (GPC3). This is a membrane glycoprotein which is involved in cell cycle regulation and which is detected in HCC patients. Although there is as yet no unanimous agreement on this, high levels of GPC3 in HCC tissue after curative resection and LT seem to lead to poor prognosis in terms of both disease free and overall survival[98,99]. In addition, a link has been found between high GPC3 expression and high tumor grade (moderate and poor differentiation), late TNM stage (III, IV), vascular invasion, tumor multifocality and metastasis in patients with HCC. Research has also shown high GPC3 expression to be associated with the presence of large tumors (5 cm or more)[100]. The importance of glypican 3 in patients with HCC undergoing liver transplantation has already been proved to be useful in prognosis stratification and several authors propose a cut-off value of 3.5 × 10-2 [101,102].

 Another important biomarker to mention is human telomerase reverse transcriptase mRNA (h-TERT mRNA). Several studies have demonstrated that high h-TERT mRNA expression is a prognostic indicator of poor outcome in HCC patients. The prognostic power of h-TERT mRNA has been evaluated also in a liver transplant setting: HCC patients with an elevation of human telomerase reverse transcriptase mRNA (preoperatively in the blood or after neoadjuvant immunochemotherapy) suffered of higher tumor recurrence and lower survival rates than those without h-TERT mRNA in the blood[103,104].More studies are required to validate the prognostic power of h-TERT mRNA due to an absence of an unanimous consensus[105,106].

High levels of alpha 1 fucosidase (AFU)and transforming growth factor beta-1 (TGF-B1) seem to be associated with poor prognosis in patients with HCC. Although more studies are required to evaluate the importance of said biomarkers after LT[107-113].

Furthermore, biomarkers such as human cervical cancer oncogene (HCCR), tumor specific growth factor (TSGF) and gamma-glutamyl transferase II (GGT II) have been proved to have an important role in diagnosis, but more studies are required to clarify its prognostic role[56,114-120].

***Systemic inflammatory markers***

Systemic host inflammation is another factor that has been evaluated as a parameter to assess tumor aggressiveness[121,122]. Depending on the tumor micro-environment, pro-inflammatory macrophages, cytokines and chemokines seem to be factors responsible for tumor progression given their ability to induce metastatization and to inhibit apoptosis, thus facilitating angiogenesis and DNA damage[123].

Neutrophil-to-lymphocyte ratio (NLR) is a serum inflammatory marker that has been attracting increasing interest since it has been found to be a predictor of recurrence and poor prognosis in patients with colorectal-liver metastasis[123-125]. High neutrophil levels are able to enhance the propensity for vascular invasion and metastatization by increasing the production of VEGF[126,127]. Conversely, low lymphocyte numbers seem to be responsible for impaired immunosurveillance against disease development and progression[128].

In 2009, Halazun *et al*[123] demonstrated that NLR predicts outcome in HCC patients after LT. They reported that patients meeting the Milan criteria with a NLR ≥ 5 had significantly worse recurrence-free survival (RFS) and lower survival rates than patients with a NLR < 5. Similar effects on tumor recurrence and survival were later reported by other groups both for cadaveric and for living donor liver transplantation (LDLT)[129-132]. A recent meta-analysis evaluating the prognostic significance of NLR in HCC patients confirmed, moreover, that high NLR was associated with poor OS and DFS of liver transplanted patients with HRs of 3.42 (2.41-4.85) and 5.90 (3.99-8.70), respectively. Notably, conventional prognostic indexes such as vascular invasion, multiple tumors, and AFP ≥ 400 ng/mL were also reported to be correlated with NLR[133]. Despite discordant findings[69,134], the data uncovered until now suggest that NLR can contribute to the LT selection process of HCC patients. Further data are needed to confirm the marker’s effectiveness.

Other inflammatory-related prognostic markers are under evaluation. In a retrospective intention-to-treat analysis on 181 HCC patients listed for LT, Lai *et al*[135] demonstrated that the platelet-to-lymphocytes ratio (PLR) > 150 was more efficacious than NLR in predicting the risk of HCC recurrence after LT and that it can be used to stratify patients for tumor-free-survival (91.6% *vs* 80.7%, *P* = 0.02). The usefulness of PLR was recently confirmed by a Chinese study focusing on a cohort of 343 HCC in whom a PLR = 125 was found to be the most appropriate cut-off to predict tumor-free survival after LT (sensitivity 61.6%, specificity 62.7%)[136]. Unanimous agreement has, however, yet to be reached[134].

Some have hypothesized that an inflammatory response is implicated in the pathogenesis of cancer-related malnutrition[137]. The Prognostic Nutritional Index (PNI) has been proposed as a further marker of inflammation and HCC-related prognosis. Chan *et al*[138]demonstrated that PNI is an independent prognostic index of OS and DFS after surgical resection of Barcelona Clinic Liver Cancer (BCLC) Stage 0/A, but its potential role in the liver transplant setting is as yet unestablished.

Due to a clear lack of single, self-sufficient prognostic biomarkers, some attempts have been made to assess prognosis using an integrated combination of more than one of these. Toyoda *et al*[139] for example, used an Asian population to develop the so-called BALAD staging score which is based on 5 serum markers including AFP, AFP-L3% and DCP; the scoring system’s predictive power was found to be similar to that of the BCLC staging system. Although the model was recently validated on a British population[140], it has yet to be validated in a transplant setting.

Retrospectively studying 185 patients who underwent hepatectomy for HCC, Kiriyama concluded that triple positive tumor markers for HCC (AFP, AFP-L3% and DCP) correlated with the poorest prognosis and the most invasive characteristics in pathological findings[141].

As a final consideration, research on miRNA plasma expression is arousing interest in view of the potential role of miRNA signature profiling in HCC prognosis stratification[142].

**RADIOLOGICAL FEATURES: THE ROLE OF 18F-FDG-PET IMAGING**

Morphological imaging studies have proven to be effective in predicting outcome after orthotopic liver transplantation (OLT). While both the Milan and the UCFS criteria are based on the size and number of radiologically detected tumors, functional imaging studies appear to be able to provide even further information about the tumor. F-18 fluoro-2-deoxy-d-glucose Positron Emission Topography (18F-FDG PET) estimates the tumor growth and metabolism based on calculated tumor volumes and maximum standardized uptake values. The different ratio of glucose-6-phosphatase and hexokinase in the normal liver and tumor cells results in an increased accumulation of 18F-FDG in primary HCC lesions[143].

While 18F-FDG-PET has demonstrated suboptimal sensitivity in detecting new HCC (< 50%)[144], there are reports that it is useful in uncovering the presence of extra-hepatic metastasis[145], in providing information about HCC prognosis, and in predicting tumor recurrence after LT[146-149]. In a retrospective analysis, Yang *et al*[146] demonstrated that 18F-FDG-PET positive patients (PET +) showed an overall greater risk of tumor recurrence with respect to PET negative (PET-) patients (OR = 7.6). Its ability to predict prognosis and tumor recurrence was confirmed in 2009 by Kornberg *et al*[147] who carried out a retrospective analysis on 42 liver transplanted patients. Their results demonstrated that PET+ patients had a significantly worse 3-ys DFS (35%) and a higher recurrence rate (RR 50%) than PET- patients (DFS = 93%, RR = 3.8%). The same research group recently demonstrated that HCC patients meeting the Milan criteria with a non-avid 18F-FDG PET achieved an excellent DFS after LT (> 80% at 5-yr follow-up). An avid uptake of 18F-FDG was found to be an independent predictor of tumor related drop-out from waiting lists. This finding confirms the potential advantages of this technique in the LT setting[148].

As far as biological findings are concerned, Yang *et al*[147] demonstrated that PET+ (greater PET lesion uptake) HCCs were significantly associated with some poor prognostic factors such as AFP > 200 ng/mL (*P* < 0.001) and vascular invasion (*P* = 0.003)[146]. Kornberg likewise demonstrated that PET+ status was an independent predictor of microvascular invasion. The uptake of 18F-FDG in HCC patients is reported, moreover, to vary according to the degree of tumor differentiation[146]. Well- and well-to-moderately differentiated HCCs, in particular, exhibit an 18F-FDG metabolism that is similar to normal liver tissue while moderate-to-poorly and poorly differentiated HCCs demonstrate an enhanced one[146,150,151].

New tracers (*e.g.,* 18F-fluoropropyloxy-L-tryptophan and L-methyl-3H-methionine, 11C-metomidate, 11C-acetate, 18F-fluorocholine and 11C-choline) aiming to improve PET’s specificity and sensitivity in detecting HCC and its metastases are presently under investigation[152-154]. Dual Tracer PET-CT imaging is also under examination: 11C-acetate and 18F-FDG have already demonstrated high sensitivity and specificity in detecting HCC (about 95% and 100% respectively) in candidates for LT or liver resection[155,156]; the potential role of these new tracers and the recently introduced oncologic PET-MRI[157] in the transplant scenario and their ability to detect biological aggressiveness remain to be established.

**HISTOLOGY: THE PRE-TRANSPLANT LIVER BIOPSY**

As mentioned above, microvascular invasion is an established independent prognostic factor for HCC recurrence after LT[2-7]. A strong correlation between microvascular invasion and the histologic tumor grade of HCC has, in fact, been reported by many authors[2,14,19,158,159] , and high tumor grade has been found to be an independent predictor of vascular invasion[160]. In addition, Tamura *et al*[42] reported that histologic differentiation itself represents an independent predictor of survival following transplantation since a low tumor grade increases the 3-year survival rate both in patients with small (≤ 5 cm) and large (> 5 cm) tumors.

A very low post-transplant tumor recurrence rate was found by our group when a pre-transplant biopsy grade-based selection protocol that did not include the patient’s Milan criteria standing was utilized in 145 HCC patients.When the G3 HCCs were excluded from LT, the 5 year survival free recurrence was 92% and none of the patients with tumors > 5 cm had recurrence[19]. When poorly differentiated HCCs were excluded from our patient database, only 12% of the tumors were > 5 cm which, of course, confirms the relevance of a tumor grading selection system. DuBay *et al*[161] who subsequently validated this approach, studied 294 HCC patients for 8 years during which time they gradually tested and developed a transplant selection protocol. Their findings showed results gradually shifting away from the Milan criteria and towards a biopsy-based system (the extended Toronto criteria). A comparison between the two periods of the study confirmed that the exclusion of poorly differentiated tumors irrespective of macromorphological features (Milan criteria)achieved excellent survival rates.

Findings from Pawlik *et al*[162] single-center study did not, however, confirm the independent prognostic power of a pre-operative biopsy for outcome after LT. While the tumor grade associated with vascular invasion or disease-specific death was not significant, the tumor grade on the final pathologic examination was found to be a reliable predictor of vascular invasion and outcome. The disparity between the preoperative histology and the final surgical specimen analysis may be explained by the fact that well or moderately-differentiated tumor areas can coexist adjacent to poorly differentiated ones. Sampling bias should, therefore, always be taken into consideration. The median tumor size in Pawlik *et al*[165] study was 7.0 cm, and half of the patients had very large tumors with a markedly increased risk of heterogeneity and dedifferentiation[163,164]. More generally, while the biopsy reading is strictly related to the quality of the tissue sample, the histologic grade is subject to inter-observer variability. Notwithstanding the existence of the Edmondson and Steiner grading system, and although automated grading systems have been proposed[166] , HCC biopsy imaging grading is still visual, qualitative, and subjective. Indeed, the inter-observer variability has been shown to be relevant, with a K statistic from 0.32 to 0.66, respectively, for moderately and well differentiated HCC[167]*.* Lack of concordance clearly limits the widespread use of a grade-based selection protocol of HCC patients for LT.

On the other hand, if one goes beyond the possibility of “false negative” results, virtually all G3 biopsies in HCC patients refer to true poorly differentiated tumors, and all high grade tumors prove to be strictly correlated with poor outcome after LT. Excluding these patients from LT listing may contribute to reducing the prevalence of patients with aggressive HCC and this, of course, will lead to beneficial results in terms of overall post-transplant outcome, as has been reported by one of our studies as well as by a Canadian study.

Multiple fine needle aspiration biopsies (FNABs) are not, however, recommended given the complication rates that vary from 0.75% to 13.6%[168]. Cases of bleeding have also been described in 1/500 biopsies and those requiring urgent hospitalization and blood transfusion range from 1 /2500 to up to 1/10000 biopsies. There is, in addition, a 0 to 3% risk of needle tract seeding in most studies although it did not seem to influence the oncological outcome[169-171].

A visual liver and biopsy site assessment could overcome these sampling issues. Core needle biopsy (CNB) under laparoscopic ultrasonography (LUS) guidance makes it possible to directly examine the area to be sampled[172,173]. Helmreich *et al*[174] described LUS guided CNB as a safe and feasible procedure. LUS appears to be extremely promising tool in the transplant setting; biopsy specimens and high diagnostic accuracy can be obtained, and HCC can be treated (downstaging – bridging) all at the same time and potentially repeatedly. Further studies are needed to establish its role in the pre-transplant assessment of HCC patients.

While current guidelines do not include liver biopsy to diagnose radiologically typical[175]HCC, given the tendency to characterize tumors focusing on their molecular features, biopsies will presumably play a key role in the near future. Large steps forward have, in fact, been made in the molecular signatures field thanks to the development of microarray technologies which permit the tumor expression of several molecular markers associated with deregulation of genes or pathways to be tested at the same time by processing a tumor tissue sample[176,177]. Deregulation of these genes and pathways has, in turn, been proven to affect some of the tumor’s biological features such as vascular invasion[178,179] and growth rate[180,181], and several studies have shown that it plays a role in worsening prognosis[181-184]. In a recent paper, Villa *et al*[181] demonstrated that a five-gene trascriptomic hepatic signature including angiopoietin-2, NETO2, DLL4 ESM1 and NR4A1 was able to rapidly identify growing HCCs and was independently associated with an increase in mortality. Several signatures, including the miRNA expression pattern[185] have been proposed and studied until now, but agreement on the best predictive molecular signature pattern has yet to be reached.

It is worth mentioning that epigenetic features have also been investigated as prognostic biomarkers. In fact, as with other cancers, HCC has demonstrated a distinct methylation profile. The hypermethylated form of p16 (CDKN2A), for example, a tumor-suppressor gene involved in cell cycle regulation, has already been associated with advanced stages of HCC, vascular invasion, poor tumor differentiation and, finally, with worse prognosis[186].

In view of its relatively recent appearance, the molecular signature still lacks clinical relevance. Evidence gathered until now suggests that it can be potentially used in the LT evaluation workup. Even more importantly, in the same way that a great deal of information can be gained from a biopsy specimen, the same can be said for the tumor’s histological features. In addition, recent technologies have made it possible to obtain genomic profiling on formalin fixed, paraffin embedded samples making the molecular signature a feasible, reproducible tool for tumor evaluation in the near future[184].

**RESPONSE TO THERAPY**

Loco-regional therapy response in LT candidates with HCC has been extensively studied over the last 10 to 15 years, but an in-depth analysis of the topic does not fall within the aims of the current work[15,29,175,187-200]*.* Despite the fact that there is a paucity of randomized clinical trials regarding downstaging or bridging therapies, most centers throughout the world adopt loco-regional therapies before or after placing a patient on a waiting list[188,196,201-203]*.*

Resection, ablation (either percutaneous or laparoscopic), or TACE alone or together with other therapies, which are the most widely used therapeutic strategies, aim to reduce the dropout rate while patients are on waiting lists and/or tumor recurrence after LT[204-206]*.* Although downstaging strategies focus on reducing the tumor burden until the patient meets transplantation criteria (*i.e.,* Milan), it is widely accepted that a prolonged response to downstaging therapies can itself be considered a selection criteria that mirrors the biologic behavior of the tumor and predicts a relatively low risk of recurrence after LT. Similarly, as reported by a number of studies, a good response to bridging therapies can serve as a surrogate marker of a favorable tumor biology (Table 2) .

In an intention-to-treat analysis, Millonig *et al*[187] demonstrated that patients with a complete response to TACE had 1-, 2-, and 5-year survival rates of 89.1, 85.1, and 85.1%, respectively, compared with 68.6, 51.4, and 51.4% in non-responders (*P* = 0.02). Similar results were also found by our group while studying recurrence after LT in patients who were stratified into responder and non-responders to pre-transplant HCC therapy. The probability of post-transplant HCC recurrence was shown to be higher in the non- responder (*P* = 0.04) group[15]. In a seminal study by Otto *et al*[207] 136 HCC patients who underwent TACE were assessed during the waiting period for LT in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The authors reported a 22% 5-year freedom from recurrence rate in the TACE-with-progression group and a 92% rate in the TACE-without-progression one (*P* < 0.0001) Response to therapy was also more predictive of outcome than was the Milan criteria (*P* = 0.0001).

Different 5-year recurrence rates (19.4% in patients with partial or no response to bridging therapy *vs* 5.5% in responders) were also detected by Cucchetti *et al*[199] who studied the data of 315 LT candidates. A strict correlation between tumor responsiveness and outcome was also found when progression or no-progression was found at the pathological examination[208,209]. Ho *et al*[210] assessed loco-regional treatment (both as downstaging or bridging strategies) before LT in 86 HCC patients by dividing the population into 3 subgroups depending on the degree of tumor necrosis at the pathological examination (group I: 10%-50%; group II: 50-90%; and group III: > 90). The patients with a higher necrosis rate after therapy (group III) were found to survive longer (*P* = 0.003) and had significantly lower recurrence rates than the patients in the other two groups (*P* = 0.001). Finally, when response to therapy was integrated with higher AFP levels, its power to predict prognosis in terms of RFS and OS was found to be enhanced. Absence of progression as far as tumor burden and the AFP (slope ≤ 15) were concerned identified a subgroup of patients with excellent prognosis, irrespective of conventional criteria[211] (Table 3).

With regard to downstaging, some studies have shown that the outcome of downstaged HCCs patients was similar to that of patients meeting the Milan criteria. Ravaioli *et al*[17]reported a 3-year RFS in patients fulfilling the Milan criteria and in downstaged patients of 83% and 75%, respectively (*P* = NS). As outlined in Table 2, similar results were found by Chapman *et al*[212] and De Luna[213]. Yao *et al*[214] recently reported long term results after LT in HCC patients downstaged to T2 and compared their data to those of patients meeting the Milan criteria: the 5-year post-transplant and recurrence-free survival were 77.8% and 90.8%, respectively, in the downstaged group versus 81% and 88% in the T2 group (*P* = NS). Since the 2-year cumulative probability for dropout was 34.2% in the downstaged group versus 25.6% in the T2 LT recipient group, this suggests that downstaging has an important impact on tumor biology. Interestingly, the prevalence of microvascular invasion and of poorly differentiated grade was found to be similar in the two groups when the final pathological features were compared.

These findings provide further evidence that non-responsive HCCs could conceal an aggressive biology while responsive ones could mirror a milder behavior. At the same time, they emphasize the relevance of response to therapy as an index of tumor biologic aggressiveness and the need to develop standardized guidelines to evaluate it. Several studies suggest using the modified version of Response Evaluation Criteria In Solid Tumours (mRECIST)[215-218] which constitute the modified version of the RECIST criteria for the assessment of response to therapy in solid tumors[219]. With respect to conventional criteria, modified ones include an oncological assessment of tumor viability and focus on reducing the viable tumor volume, defined by enhanced areas on imaging. Standardized criteria could help to improve homogeneity in response to therapy assessments.

There are, nevertheless, still some limitations with regard to the reliability and reproducibility of mRECIST. Since tumor assessment is subjective, its accuracy depends both on the technician’s ability as well as on the quality of imaging[220]. Furthermore, its criteria are only applicable to HCC with typical features because assessment of response in atypical HCC remains obscure[220]. Finally, vascular shunt and alterations (especially for infiltrative tumors) can alter enhancement and thus lead to interpretation errors. In view of these and other considerations, some authors suggest evaluating AFP variations together with radiological features for an objective assessment of response to therapy[47,211].

A potential drawback of bridging and downstaging therapies is, however, tumor dedifferentiation. Kojiro *et al*[221] reported that tumors with sarcomatoid changes were more frequent in patients who were treated with TACE before transplant than those who were not. Sarcomatoid modification has also been described in some case reports after radiofrequency ablation[222,223]. Zen *et al*[224] reported that only pretreated HCCs showed dedifferentiation towards a biliary phenotype when they analyzed explanted specimens. Yamamoto *et al*[225] reported a high recurrence rate after radiofrequency ablation (RFA) in tumors that had aggressive biological features.

Selection pressure on resistant cells, phenotypic adaptative changes, and the protein expression normally trigged by hypoxia may explain therapy-induced histopathological changes[224,226]. Regardless of etiology, caution should be used when applying pre-LT therapies as investigation tools given the biological switch phenomenon.

***Test of time***

A complete or partial response to therapy does not guarantee that it will be stable over time, and a rapid recurrence after response could uncover an aggressive tumor biology which would contraindicate the transplant. Not only the assessment of the response to therapy but also the course following that response (or more generally the overall tumor growth rate) are important considerations when HCC patients are being evaluated for LT as far as the so-called “test of time" is concerned. Reporting good outcomes when tumors were closesly evaluated for 8 mo from the time of ablation to the date of enlisting a patient for transplant[210] , Roberts *et al*[210] proposed scheduling a waiting period following downstaging procedures to assess tumor behavior. Toso *et al*[227] also stressed the importance of an observation period between entering a downstaging program and being placed on a transplantation waiting list and suggested utilizing at least a 6-month minimum test of time. Cescon *et al*[122] instead, proposed using a 3-month waiting period with re-staging at the end to verify the new status. Despite the drawback of having to wait and the intrinsic increase in drop-out risk, the test of time appears to be an efficacious surrogate marker of tumor aggressiveness that could be integrated with other “static” prognostic tools (histology, response to therapy, morphologic studies). Further studies using standardized response assessments and homogeneous periods are needed to evaluate the parameter’s true potential.

**VOLUME DOUBLING TIME**

In 1961 Mordecai Schwartz proposed a biomathematical approach to clinical tumor growth and the formula he outlined to calculate the oubling time (DT) was:

*DT=t ln 2/(ln V2−ln V1)*

Where *t* is the time interval between measurements and *V2* and *V1* are the tumor volumes detected at imaging, respectively, at the end and at beginning of the time interval[228].

Subsequent studies described a wide variability in DTs with values ranging from < 30 d up to 600 d[229-234]. The assumption that various growth velocities reflect different tumor behaviors led researchers to search for correlations between HCC DT and other tumor or patient characteristics. The close relationship between DTs and prognosis has recently been investigated by Villa *et al*[181] in a prospective study on 78 patients with newly diagnosed HCC. Study data confirmed that tumor doubling time ranged from 30 to 621 d. When the study population was divided into quartiles according to HCC growth rate, different survival profiles depending on the speed of tumor DT were found: 25% of the patients demonstrated DTs less than or equal to 53 d and had a significantly worse prognosis than the patients with DTs in other quartiles, regardless of the treatment prescribed[181].

Static macromorphological parameters such as initial tumor diameter and ultrasound features have been demonstrated to be correlated with DT[231,234]. More interestingly, however, DT seems to be correlated with tumor differentiation[229,231,232]*,* mitotic activity[229,230], vascular invasion and direct indexes of biological aggressiveness. Nakajima *et al*[229] studied 34 patients with small HCCs using some markers of cell division such as Ki-67, Apo-I and their histologic grade to classify the tumors as slowly, moderately, or rapidly growing. They concluded that the more rapid the tumor growth, the higher the cell production and the less differentiated the tumor[229]. Moreover, as reported by several studies[232,234-236], AFP levels were found to be correlated with tumor growth velocity, and this finding has confirmed the indirect link between DT and biological aggressiveness.

In addition, the direct influence of DT on outcome after surgery has also been reported. Okazaki *et al*[230] outlined poor outcomes after hepatectomy in those patients whose DT was short*.* Similarly, Cucchetti *et al*[232] calculated that the 3-year recurrence rate after liver resection was significantly higher in patients with DT < 100 d than in those with DT > 100 d (*P* = 0.008). Even if there are no reports on the effect of DT on the post-transplant outcome, the studies mentioned above clearly demonstrate that a tumor’s growth velocity is a faithful mirror of its intrinsic aggressiveness. It seems reasonable then, although there are no studies to prove it, that rapidly growing tumors have poor outcomes after LT.

While the strict correlation between AFP levels and tumor growth velocity has been repeatedly demonstrated[232,234-236], the lack of agreement about this link[237] and the ease of obtaining the DT parameter point the way to further research on the role of DT in the evaluation of HCC aggressiveness in LT candidates.

**CONCLUSION**

Predicting post-transplant HCC recurrence on the basis of the tumor size and the number of nodules can only seem simplistic and imprecise in the light of the disease’s complexity. A number of recent studies have confirmed the predictive accuracy of other parameters used to assess the biological behavior of HCC and in particular with reference to tumor progression and response to therapy. Prospective randomized studies designed to validate the prognostic role of each of these parameters present relevant feasibility issues. Repeatable, multiparametric, integrated models develped on the basis of large multicentric prognostic studies are no doubt the best strategy to improve our ability to select the most appropriate HCC patients for liver transplant.

**REFERENCES**

1 **Freeman RB**, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* 2008; **8**: 958-976 [PMID: 18336699 DOI: 10.1111/j.1600-6143.2008.02174.x]

2 **Jonas S**, Bechstein WO, Steinmüller T, Herrmann M, Radke C, Berg T, Settmacher U, Neuhaus P. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. *Hepatology* 2001; **33**: 1080-1086 [PMID: 11343235 DOI: 10.1053/jhep.2001.23561]

3 **Hemming AW**, Cattral MS, Reed AI, Van Der Werf WJ, Greig PD, Howard RJ. Liver transplantation for hepatocellular carcinoma. *Ann Surg* 2001; **233**: 652-659 [PMID: 11323504 DOI: 10.1097/00000658-200105000-00009]

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

5 **Marsh JW**, Dvorchik I, Subotin M, Balan V, Rakela J, Popechitelev EP, Subbotin V, Casavilla A, Carr BI, Fung JJ, Iwatsuki S. The prediction of risk of recurrence and time to recurrence of hepatocellular carcinoma after orthotopic liver transplantation: a pilot study. *Hepatology* 1997; **26**: 444-450 [PMID: 9252157 DOI: 10.1002/hep.510260227]

6 **Margarit C**, Charco R, Hidalgo E, Allende H, Castells L, Bilbao I. Liver transplantation for malignant diseases: selection and pattern of recurrence. *World J Surg* 2002; **26**: 257-263 [PMID: 11865357 DOI: 10.1007/s00268-001-0214-1]

7 **Iwatsuki S**, Dvorchik I, Marsh JW, Madariaga JR, Carr B, Fung JJ, Starzl TE. Liver transplantation for hepatocellular carcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 2000; **191**: 389-394 [PMID: 11030244 DOI: 10.1016/S1072-7515(00)00688-8]

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

9 **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**: 2587-2596 [PMID: 17868066 DOI: 10.1111/j.1600-6143.2007.01965.x]

10 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]

11 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

12 **Sotiropoulos GC**, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki E, Kühl H, Dirsch O, Lang H, Broelsch CE. Liver transplantation for hepatocellular carcinoma in cirrhosis: is clinical tumor classification before transplantation realistic? *Transplantation* 2005; **79**: 483-487 [PMID: 15729176 DOI: 10.1097/01.TP.0000152801.82734.74]

13 **Roayaie S**, Frischer JS, Emre SH, Fishbein TM, Sheiner PA, Sung M, Miller CM, Schwartz ME. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. *Ann Surg* 2002; **235**: 533-539 [PMID: 11923610 DOI: 10.1097/00000658-200204000-00012]

14 **Klintmalm GB**. Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. *Ann Surg* 1998; **228**: 479-490 [PMID: 9790338 DOI: 10.1097/00000658-199810000-00005]

15 **Vitale A**, D'Amico F, Frigo AC, Grigoletto F, Brolese A, Zanus G, Neri D, Carraro A, D'Amico FE, Burra P, Russo F, Angeli P, Cillo U. Response to therapy as a criterion for awarding priority to patients with hepatocellular carcinoma awaiting liver transplantation. *Ann Surg Oncol* 2010; **17**: 2290-2302 [PMID: 20217249 DOI: 10.1245/s10434-010-0993-4]

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

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

18 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]

19 **Cillo U**, Vitale A, Bassanello M, Boccagni P, Brolese A, Zanus G, Burra P, Fagiuoli S, Farinati F, Rugge M, D'Amico DF. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. *Ann Surg* 2004; **239**: 150-159 [PMID: 14745321 DOI: 10.1097/01.sla.0000109146.72827.76]

20 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-94.e3; quiz e14-5 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]

21 **ABELEV GI**, PEROVA SD, KHRAMKOVA NI, POSTNIKOVA ZA, IRLIN IS. Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation* 1963; **1**: 174-180 [PMID: 14010646 DOI: 10.1097/00007890-196301020-00004]

22 **Alpert E**, Hershberg R, Schur PH, Isselbacher KJ. -fetoprotein in human hepatoma: improved detection in serum, and quantitative studies using a new sensitive technique. *Gastroenterology* 1971; **61**: 137-143 [PMID: 4104961]

23 **Kew M**. Alpha-fetoprotein in primary liver cancer and other diseases. *Gut* 1974; **15**: 814-821 [PMID: 4140084 DOI: 10.1136/gut.15.10.814]

24 **Chen DS**, Sung JL. Serum alphafetoprotein in hepatocellular carcinoma. *Cancer* 1977; **40**: 779-783 [PMID: 70268 DOI: 3.0.CO; 2-Y']

25 **Johnson PJ**, Portmann B, Williams R. Alpha-fetoprotein concentrations measured by radioimmunoassay in diagnosing and excluding hepatocellular carcinoma. *Br Med J* 1978; **2**: 661-663 [PMID: 81086 DOI: 10.1136/bmj.2.6138.661]

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

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

28 **Han K**, Tzimas GN, Barkun JS, Metrakos P, Tchervenkov JL, Hilzenrat N, Wong P, Deschênes M. Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007; **21**: 39-45 [PMID: 17225881]

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

30 **Todo S**, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: S48-S54 [PMID: 17969069 DOI: 10.1002/lt.21334]

31 **Berry K**, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013; **19**: 634-645 [PMID: 23536495 DOI: 10.1002/lt.23652]

32 **Mailey B**, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, Bhatia S, Nissen N, Colquhoun SD, Kim J. Evaluation of absolute serum α-fetoprotein levels in liver transplant for hepatocellular cancer. *Arch Surg* 2011; **146**: 26-33 [PMID: 21242442 DOI: 10.1001/archsurg.2010.295]

33 **Xiao L**, Fu ZR, Ding GS, Fu H, Ni ZJ, Wang ZX, Shi XM, Guo WY. Liver transplantation for hepatitis B virus-related hepatocellular carcinoma: one center's experience in China. *Transplant Proc* 2009; **41**: 1717-1721 [PMID: 19545714 DOI: 10.1016/j.transproceed.2009.03.058.]

34 **Yang Y**, Nagano H, Ota H, Morimoto O, Nakamura M, Wada H, Noda T, Damdinsuren B, Marubashi S, Miyamoto A, Takeda Y, Dono K, Umeshita K, Nakamori S, Wakasa K, Sakon M, Monden M. Patterns and clinicopathologic features of extrahepatic recurrence of hepatocellular carcinoma after curative resection. *Surgery* 2007; **141**: 196-202 [PMID: 17263976 DOI: 10.1016/j.surg.2006.06.033]

35 **Wang ZX**, Song SH, Teng F, Wang GH, Guo WY, Shi XM, Ma J, Wu YM, Ding GS, Fu ZR. A single-center retrospective analysis of liver transplantation on 255 patients with hepatocellular carcinoma. *Clin Transplant* 2010; **24**: 752-757 [PMID: 20030683 DOI: 10.1111/j.1399-0012.2009.01172.x]

36 **Onaca N**, Davis GL, Jennings LW, Goldstein RM, Klintmalm GB. Improved results of transplantation for hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. *Liver Transpl* 2009; **15**: 574-580 [PMID: 19479800 DOI: 10.1002/lt.21738]

37 **Sotiropoulos GC**, Lang H, Nadalin S, Neuhäuser M, Molmenti EP, Baba HA, Paul A, Saner FH, Weber F, Hilgard P, Frilling A, Broelsch CE, Malagó M. Liver transplantation for hepatocellular carcinoma: University Hospital Essen experience and metaanalysis of prognostic factors. *J Am Coll Surg* 2007; **205**: 661-675 [PMID: 17964442 DOI: 10.1016/j.jamcollsurg.2007.05.023]

38 **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**: 874-881 [PMID: 19453644 DOI: 10.1111/j.1399-0012.2009.00993.x]

39 **Adler M**, De Pauw F, Vereerstraeten P, Fancello A, Lerut J, Starkel P, Van Vlierberghe H, Troisi R, Donckier V, Detry O, Delwaide J, Michielsen P, Chapelle T, Pirenne J, Nevens F. Outcome of patients with hepatocellular carcinoma listed for liver transplantation within the Eurotransplant allocation system. *Liver Transpl* 2008; **14**: 526-533 [PMID: 18383082 DOI: 10.1002/lt.21399]

40 **Pérez-Saborido B**, de los Galanes SJ, Menéu-Díaz JC, Romero CJ, Elola-Olaso AM, Suárez YF, Valencia VB, Moreno-González E. Tumor recurrence after liver transplantation for hepatocellular carcinoma: recurrence pathway and prognostic factors. *Transplant Proc* 2007; **39**: 2304-2307 [PMID: 17889172]

41 **Fujiki M**, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]

42 **Tamura S**, Kato T, Berho M, Misiakos EP, O'Brien C, Reddy KR, Nery JR, Burke GW, Schiff ER, Miller J, Tzakis AG. Impact of histological grade of hepatocellular carcinoma on the outcome of liver transplantation. *Arch Surg* 2001; **136**: 25-30; discussion 31 [PMID: 11146770 DOI: 10.1001/archsurg.136.1.25]

43 **Varona MA**, Soriano A, Aguirre-Jaime A, Garrido S, Oton E, Diaz D, Portero J, Bravo P, Barrera MA, Perera A. Risk factors of hepatocellular carcinoma recurrence after liver transplantation: accuracy of the alpha-fetoprotein model in a single-center experience. *Transplant Proc* 2015; **47**: 84-89 [PMID: 25645778 DOI: 10.1016/j.transproceed.2014.12.013]

44 **Notarpaolo A,** Bizouard G, Gambato M, Montalti R, Magini G, Miglioresi L, Vitale A, Vennarecci G, Ambrosio CD, Burra P. Prediction of Recurrence after Liver Transplantation for HCC: Validation of the AFP Model in an Italian Cohort: Wiley-blackwell 111 River st, Hoboken 07030-5774, NJ, USA, 2014: S132-S132 [DOI: 10.1016/S0168-8278(14)61076-X ]

45 **Hameed B**, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level & gt; 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014; **20**: 945-951 [PMID: 24797281 DOI: 10.1002/lt.23904]

46 **Kondili LA**, Lala A, Gunson B, Hubscher S, Olliff S, Elias E, Bramhall S, Mutimer D. Primary hepatocellular cancer in the explanted liver: outcome of transplantation and risk factors for HCC recurrence. *Eur J Surg Oncol* 2007; **33**: 868-873 [PMID: 17258882 DOI: 10.1016/S0168-8278(04)90255-3]

47 **Merani S**, Majno P, Kneteman NM, Berney T, Morel P, Mentha G, Toso C. The impact of waiting list alpha-fetoprotein changes on the outcome of liver transplant for hepatocellular carcinoma. *J Hepatol* 2011; **55**: 814-819 [PMID: 21334400 DOI: 10.1016/j.jhep.2010.12.040]

48 **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**: 987-999 [PMID: 22429190 DOI: 10.1111/j.1365-2036.2012.05060.x]

49 **Debruyne EN**, Delanghe JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. *Clin Chim Acta* 2008; **395**: 19-26 [PMID: 18538135 DOI: 10.1016/j.cca.2008.05.010]

50 **Yang GH**, Fan J, Xu Y, Qiu SJ, Yang XR, Shi GM, Wu B, Dai Z, Liu YK, Tang ZY, Zhou J. Osteopontin combined with CD44, a novel prognostic biomarker for patients with hepatocellular carcinoma undergoing curative resection. *Oncologist* 2008; **13**: 1155-1165 [PMID: 18997126 DOI: 10.1634/theoncologist.2008-0081]

51 **Zhang XF**, Qi X, Meng B, Liu C, Yu L, Wang B, Lv Y. Prognosis evaluation in alpha-fetoprotein negative hepatocellular carcinoma after hepatectomy: comparison of five staging systems. *Eur J Surg Oncol* 2010; **36**: 718-724 [PMID: 20538423 DOI: 10.1016/j.ejso.2010.05.022]

52 **Liebman HA**, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; **310**: 1427-1431 [PMID: 6201741 DOI: 10.1056/NEJM198405313102204]

53 **Fujiyama S**, Morishita T, Sagara K, Sato T, Motohara K, Matsuda I. Clinical evaluation of plasma abnormal prothrombin (PIVKA-II) in patients with hepatocellular carcinoma. *Hepatogastroenterology* 1986; **33**: 201-205 [PMID: 2433199]

54 **Fujiyama S**, Morishita T, Hashiguchi O, Sato T. Plasma abnormal prothrombin (des-gamma-carboxy prothrombin) as a marker of hepatocellular carcinoma. *Cancer* 1988; **61**: 1621-1628 [PMID: 2450634 DOI: 3.0.CO; 2-C']

55 **Okuda H**, Obata H, Nakanishi T, Furukawa R, Hashimoto E. Production of abnormal prothrombin (des-gamma-carboxy prothrombin) by hepatocellular carcinoma. A clinical and experimental study. *J Hepatol* 1987; **4**: 357-363 [PMID: 3036940 DOI: 10.1016/S0168-8278(87)80546-9]

56 **Zhou L**, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 1175-1181 [PMID: 16534867]

57 **Mita Y**, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 1998; **82**: 1643-1648 [PMID: 9576283 DOI: 3.0.CO; 2-B']

58 **Tanaka Y**, Kashiwagi T, Tsutsumi H, Nagasawa M, Toyama T, Ozaki S, Naito M, Ishibashi K, Azuma M. Sensitive measurement of serum abnormal prothrombin (PIVKA-II) as a marker of hepatocellular carcinoma. *Hepatogastroenterology* 1999; **46**: 2464-2468 [PMID: 10522021]

59 **Ikoma J**, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, Iwasa M, Tamaki S, Watanabe S, Adachi Y. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. *Hepatogastroenterology* 2002; **49**: 235-238 [PMID: 11941963]

60 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121 [PMID: 12717392 DOI: 10.1053/jhep.2003.50195]

61 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]

62 **Poté N**, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, Puy H, Bedossa P, Paradis V. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015; **62**: 848-854 [PMID: 25450201 DOI: 10.1016/j.jhep.2014.11.005]

63 **Ishii M**, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, Toyota T, Takahashi T, Kasukawa R. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. *Am J Gastroenterol* 2000; **95**: 1036-1040 [PMID: 10763956]

64 **Hamamura K**, Shiratori Y, Shiina S, Imamura M, Obi S, Sato S, Yoshida H, Omata M. Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; **88**: 1557-1564 [PMID: 10738213 DOI: 3.0.CO; 2-G']

65 **Kobayashi M**, Ikeda K, Kawamura Y, Yatsuji H, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Kumada H. High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; **115**: 571-580 [PMID: 19117347 DOI: 19117347]']

66 **Imamura H**, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, Makuuchi M, Kawasaki S. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg* 1999; **86**: 1032-1038 [PMID: 10460639 DOI: 10.1046/j.1365-2168.1999.01185.x]

67 **Sakaguchi T**, Suzuki S, Morita Y, Oishi K, Suzuki A, Fukumoto K, Inaba K, Nakamura S, Konno H. Impact of the preoperative des-gamma-carboxy prothrombin level on prognosis after hepatectomy for hepatocellular carcinoma meeting the Milan criteria. *Surg Today* 2010; **40**: 638-645 [PMID: 20582515 DOI: 10.1007/s00595-009-4109-3]

68 **Toyoda H**, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, Oka H, Yamazaki O, Manabe T, Urano F, Chung H, Kudo M, Matsunaga T. Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; **49**: 223-232 [PMID: 18571271 DOI: 18571271]']

69 **Shindoh J**, Sugawara Y, Nagata R, Kaneko J, Tamura S, Aoki T, Sakamoto Y, Hasegawa K, Tanaka T, Kokudo N. Evaluation methods for pretransplant oncologic markers and their prognostic impacts in patient undergoing living donor liver transplantation for hepatocellular carcinoma. *Transpl Int* 2014; **27**: 391-398 [PMID: 24472068 DOI: 10.1111/tri.12274]

70 **Taketomi A**, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, Harada N, Yamashita Y, Sugimachi K, Kayashima H, Iguchi T, Maehara Y. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009; **87**: 531-537 [PMID: 19307789 DOI: 10.1097/TP.0b013e3181943bee]

71 **Chaiteerakij R**, Zhang X, Addissie BD, Mohamed EA, Harmsen WS, Theobald PJ, Peters BE, Balsanek JG, Ward MM, Giama NH, Moser CD, Oseini AM, Umeda N, Venkatesh S, Harnois DM, Charlton MR, Yamada H, Satomura S, Algeciras-Schimnich A, Snyder MR, Therneau TM, Roberts LR. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. *Liver Transpl* 2015; **21**: 599-606 [PMID: 25789635 DOI: 25789635]']

72 **Inagaki Y**, Xu HL, Hasegawa K, Aoki T, Beck Y, Sugawara Y, Tang W, Kokudo N. Des-gamma-carboxyprothrombin in patients with hepatocellular carcinoma and liver cirrhosis. *J Dig Dis* 2011; **12**: 481-488 [PMID: 22118699 DOI: 10.1111/j.1751-2980.2011.00521.x]

73 **Tang W**, Miki K, Kokudo N, Sugawara Y, Imamura H, Minagawa M, Yuan LW, Ohnishi S, Makuuchi M. Des-gamma-carboxy prothrombin in cancer and non-cancer liver tissue of patients with hepatocellular carcinoma. *Int J Oncol* 2003; **22**: 969-975 [PMID: 12684661 DOI: 10.3892/ijo.22.5.969]

74 **Tang W**, Kokudo N, Sugawara Y, Guo Q, Imamura H, Sano K, Karako H, Qu X, Nakata M, Makuuchi M. Des-gamma-carboxyprothrombin expression in cancer and/or non-cancer liver tissues: association with survival of patients with resectable hepatocellular carcinoma. *Oncol Rep* 2005; **13**: 25-30 [PMID: 15583797 DOI: 10.3892/or.13.1.25]

75 **Suehiro T**, Matsumata T, Itasaka H, Taketomi A, Yamamoto K, Sugimachi K. Des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. *Surgery* 1995; **117**: 682-691 [PMID: 7539944 DOI: 10.1016/S0039-6060(95)80013-1]

76 **Miyaaki H**, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 962-968 [PMID: 18085353 DOI: 18085353]

77 **Suzuki M**, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, Koike K, Takaki A, Shiratori Y. Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. *J Biol Chem* 2005; **280**: 6409-6415 [PMID: 15582995 DOI: 10.1074/jbc.M406714200]

78 **Fujikawa T**, Shiraha H, Ueda N, Takaoka N, Nakanishi Y, Matsuo N, Tanaka S, Nishina S, Suzuki M, Takaki A, Sakaguchi K, Shiratori Y. Des-gamma-carboxyl prothrombin-promoted vascular endothelial cell proliferation and migration. *J Biol Chem* 2007; **282**: 8741-8748 [PMID: 17255102 DOI: M609358200]

79 **Wang SB**, Cheng YN, Cui SX, Zhong JL, Ward SG, Sun LR, Chen MH, Kokudo N, Tang W, Qu XJ. Des-gamma-carboxy prothrombin stimulates human vascular endothelial cell growth and migration. *Clin Exp Metastasis* 2009; **26**: 469-477 [PMID: 19263229 DOI: 10.1007/s10585-009-9246-y]

80 **Gao FJ**, Cui SX, Chen MH, Cheng YN, Sun LR, Ward SG, Kokudo N, Tang W, Qu XJ. Des-gamma-carboxy prothrombin increases the expression of angiogenic factors in human hepatocellular carcinoma cells. *Life Sci* 2008; **83**: 815-820 [PMID: 18976674 DOI: 10.1016/j.lfs.2008.10.003]

81 **Funaki NO**, Tanaka J, Seto SI, Kasamatsu T, Kaido T, Imamura M. Hematogenous spreading of hepatocellular carcinoma cells: possible participation in recurrence in the liver. *Hepatology* 1997; **25**: 564-568 [PMID: 9049199 DOI: 10.1002/hep.510250312]

82 **Yao F**, Guo JM, Xu CF, Lou YL, Xiao BX, Zhou WH, Chen J, Hu YR, Liu Z, Hong GF. Detecting AFP mRNA in peripheral blood of the patients with hepatocellular carcinoma, liver cirrhosis and hepatitis. *Clin Chim Acta* 2005; **361**: 119-127 [PMID: 15993394 DOI: 10.1016/j.cccn.2005.05.005]

83 **Cillo U**, Vitale A, Navaglia F, Basso D, Montin U, Bassanello M, D'Amico F, Ciarleglio FA, Brolese A, Zanus G, De Pascale V, Plebani M, D'Amico DF. Role of blood AFP mRNA and tumor grade in the preoperative prognostic evaluation of patients with hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6920-6925 [PMID: 16437593]

84 **Marubashi S**, Dono K, Nagano H, Sugita Y, Asaoka T, Hama N, Miyamoto A, Takeda Y, Umeshita K, Monden M. Detection of AFP mRNA-expressing cells in the peripheral blood for prediction of HCC recurrence after living donor liver transplantation. *Transpl Int* 2007; **20**: 576-582 [PMID: 17425725 DOI: 10.1111/j.1432-2277.2007.00480.x]

85 **Ijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860 [PMID: 11915031 DOI: 10.1053/jhep.2002.32100]

86 **Toso C**, Mentha G, Majno P. Liver transplantation for hepatocellular carcinoma: five steps to prevent recurrence. *Am J Transplant* 2011; **11**: 2031-2035 [PMID: 21831154 DOI: 10.1111/j.1600-6143.2011.03689.x]

87 **Kuromatsu R**, Tanaka M, Tanikawa K. Serum alpha-fetoprotein and lens culinaris agglutinin-reactive fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Liver* 1993; **13**: 177-182 [PMID: 7690873 DOI: 10.1111/j.1600-0676.1993.tb00627.x]

88 **Taketa K**, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, Satomura S, Matsuura S, Kawai T, Hirai H. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993; **53**: 5419-5423 [PMID: 7693340]

89 **Taketa K**. Alpha-fetoprotein: reevaluation in hepatology. *Hepatology* 1990; **12**: 1420-1432 [PMID: 1701754 DOI: 10.1002/hep.1840120625]

90 **Sato Y**, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; **328**: 1802-1806 [PMID: 7684823 DOI: 10.1056/NEJM199306243282502]

91 **Song BC**, Suh DJ, Yang SH, Lee HC, Chung YH, Sung KB, Lee YS. Lens culinaris agglutinin-reactive alpha-fetoprotein as a prognostic marker in patients with hepatocellular carcinoma undergoing transcatheter arterial chemoembolization. *J Clin Gastroenterol* 2002; **35**: 398-402 [PMID: 12394228 DOI: 10.1097/00004836-200211000-00008]

92 **Kobayashi M**, Hosaka T, Ikeda K, Seko Y, Kawamura Y, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Kumada H. Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre- and postoperatively. *Hepatol Res* 2011; **41**: 1036-1045 [PMID: 21883741 DOI: 10.1111/j.1872-034X.2011.00858.x]

93 **Beppu T**, Sugimoto K, Shiraki K, Tameda M, Kusagawa S, Nojiri K, Tanaka J, Yamamoto N, Takei Y, Takaki H, Uraki J, Nakatsuka A, Yamakado K, Takeda K. Clinical significance of tumor markers in detection of recurrent hepatocellular carcinoma after radiofrequency ablation. *Int J Mol Med* 2010; **26**: 425-433 [PMID: 20664960]

94 **Saito Y**, Shimada M, Utsunomiya T, Morine Y, Imura S, Ikemoto T, Mori H, Hanaoka J, Yamada S, Asanoma M. Prediction of recurrence of hepatocellular carcinoma after curative hepatectomy using preoperative Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein. *Hepatol Res* 2012; **42**: 887-894 [PMID: 22524419 DOI: 10.1111/j.1872-034X.2012.01004.x]

95 **Kobayashi M**, Kuroiwa T, Suda T, Tamura Y, Kawai H, Igarashi M, Fukuhara Y, Aoyagi Y. Fucosylated fraction of alpha-fetoprotein, L3, as a useful prognostic factor in patients with hepatocellular carcinoma with special reference to low concentrations of serum alpha-fetoprotein. *Hepatol Res* 2007; **37**: 914-922 [PMID: 17610501 DOI: 10.1111/j.1872-034X.2007.00147.x]

96 **Nouso K**, Kobayashi Y, Nakamura S, Kobayashi S, Takayama H, Toshimori J, Kuwaki K, Hagihara H, Onishi H, Miyake Y, Ikeda F, Shiraha H, Takaki A, Iwasaki Y, Kobashi H, Yamamoto K. Prognostic importance of fucosylated alpha-fetoprotein in hepatocellular carcinoma patients with low alpha-fetoprotein. *J Gastroenterol Hepatol* 2011; **26**: 1195-1200 [PMID: 21410750 DOI: 10.1111/j.1440-1746.2011.06720.x]

97 **Kusaba T**. Relationship between Lens culinaris agglutinin reactive alpha-fetoprotein and biological features of hepatocellular carcinoma. *Kurume Med J* 1998; **45**: 113-120 [PMID: 9658760 DOI: 10.2739/kurumemedj.45.113]

98 **Pan C**, Wang X, Chen W, Tao C, Xu X, Jin L, Chen Y, Zhu L, Zhou L, Pan Z. Reevaluation of glypican-3 as a prognostic marker in HCC using X-tile software. *Med Oncol* 2015; **32**: 359 [PMID: 25432695 DOI: 10.1007/s12032-014-0359-z]

99 **Fu SJ**, Qi CY, Xiao WK, Li SQ, Peng BG, Liang LJ. Glypican-3 is a potential prognostic biomarker for hepatocellular carcinoma after curative resection. *Surgery* 2013; **154**: 536-544 [PMID: 23601901 DOI: 10.1016/j.surg.2013.02.014]

100 **Xiao WK**, Qi CY, Chen D, Li SQ, Fu SJ, Peng BG, Liang LJ. Prognostic significance of glypican-3 in hepatocellular carcinoma: a meta-analysis. *BMC Cancer* 2014; **14**: 104 [PMID: 24548704 DOI: 10.1186/1471-2407-14-104]

101 **Wang YL**, Zhu ZJ, Teng DH, Yao Z, Gao W, Shen ZY. Glypican-3 expression and its relationship with recurrence of HCC after liver transplantation. *World J Gastroenterol* 2012; **18**: 2408-2414 [PMID: 22654434 DOI: 10.3748/wjg.v18.i19.2408]

102 **Li J**, Gao JZ, Du JL, Wei LX. Prognostic and clinicopathological significance of glypican-3 overexpression in hepatocellular carcinoma: a meta-analysis. *World J Gastroenterol* 2014; **20**: 6336-6344 [PMID: 24876756 DOI: 10.3748/wjg.v20.i20.6336]

103 **Oya H**, Sato Y, Yamamoto S, Nakatsuka H, Kobayashi T, Hara Y, Waguri N, Suda T, Aoyagi Y, Hatakeyama K. Comparison between human-telomerase reverse transcriptase mRNA and alpha-fetoprotein mRNA as a predictive value for recurrence of hepatocellular carcinoma in living donor liver transplantation. *Transplant Proc* 2006; **38**: 3636-3639 [PMID: 17175353 DOI: 10.1016/j.transproceed.2006.10.172]

104 **Sato Y**, Yamamoto S, Oya H, Nakatsuka H, Kobayashi T, Takeishi T, Hirano K, Hara Y, Watanabe T, Waguri N, Suda T, Ichida T, Aoyagi Y, Hatakeyama K. Preoperative human-telomerase reverse transcriptase mRNA in peripheral blood and tumor recurrence in living-related liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2005; **52**: 1325-1328 [PMID: 16201066]

105 **Kong SY**, Park JW, Kim JO, Lee NO, Lee JA, Park KW, Hong EK, Kim CM. Alpha-fetoprotein and human telomerase reverse transcriptase mRNA levels in peripheral blood of patients with hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2009; **135**: 1091-1098 [PMID: 19184104 DOI: 10.1007/s00432-009-0549-9]

106 **Kim YD**, Hwang S, Lee YJ, Kim KH, Ahn CS, Park KM, Moon DB, Ha TY, Song GW, Jung DH, Park SR, Hong HN, Lee SG. Preoperative peripheral blood human telomerase reverse transcriptase mRNA concentration is not a prognostic factor for resection of hepatocellular carcinoma. *Hepatogastroenterology* 2012; **59**: 1512-1515 [PMID: 22683968 DOI: 10.5754/hge10342]

107 **Zhou Y**, Ma X, Wu J, Zhang C, Wang B, Song B, Guo W, Pan B. [Preoperative serum α-1-fucosidase as an early-recurrent indicator for hepatocellular carcinoma following curative resection]. *Zhonghua Yi Xue Za Zhi* 2014; **94**: 3623-3628 [PMID: 25622951]

108 **Wang K**, Guo W, Li N, Shi J, Zhang C, Lau WY, Wu M, Cheng S. Alpha-1-fucosidase as a prognostic indicator for hepatocellular carcinoma following hepatectomy: a large-scale, long-term study. *Br J Cancer* 2014; **110**: 1811-1819 [PMID: 24569461 DOI: 10.1038/bjc.2014.102]

109 **Dituri F**, Serio G, Filannino D, Mascolo A, Sacco R, Villa E, Giannelli G. Circulating TGF-β1-related biomarkers in patients with hepatocellular carcinoma and their association with HCC staging scores. *Cancer Lett* 2014; **353**: 264-271 [PMID: 25088578 DOI: 10.1016/j.canlet.2014.07.029]

110 **Bedossa P**, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta 1 receptors in normal, cirrhotic, and neoplastic human livers. *Hepatology* 1995; **21**: 760-766 [PMID: 7875675]

111 **Ji F**, Fu SJ, Shen SL, Zhang LJ, Cao QH, Li SQ, Peng BG, Liang LJ, Hua YP. The prognostic value of combined TGF-β1 and ELF in hepatocellular carcinoma. *BMC Cancer* 2015; **15**: 116 [PMID: 25880619 DOI: 10.1186/s12885-015-1127-y]

112 **Giannelli G**, Mazzocca A, Fransvea E, Lahn M, Antonaci S. Inhibiting TGF-β signaling in hepatocellular carcinoma. *Biochim Biophys Acta* 2011; **1815**: 214-223 [PMID: 21129443 DOI: 10.1016/j.bbcan.2010.11.004]

113 **Dong ZZ**, Yao DF, Yao M, Qiu LW, Zong L, Wu W, Wu XH, Yao DB, Meng XY. Clinical impact of plasma TGF-beta1 and circulating TGF-beta1 mRNA in diagnosis of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 288-295 [PMID: 18522884]

114 **Wang L**, Yao M, Dong Z, Zhang Y, Yao D. Circulating specific biomarkers in diagnosis of hepatocellular carcinoma and its metastasis monitoring. *Tumour Biol* 2014; **35**: 9-20 [PMID: 24006223 DOI: 10.1007/s13277-013-1141-0]

115 **Farinati F,** Giacomin A. Marcatori bioumorali e molecolari dell’epatocarcinoma RIMeL – IJLaM. MAF Servizi srl ed. 2008: 2

116 **Zhu J, J**iang F, Ni HB, Xiao MB, Chen BY, Ni WK, Lu CH, Ni RZ. Combined analysis of serum γ-glutamyl transferase isoenzyme II, α-L-fucosidase and α-fetoprotein detected using a commercial kit in the diagnosis of hepatocellular carcinoma. *Exp Ther Med* 2013; **5**: 89-94 [PMID: 23251247]

117 **Yoon SK**, Lim NK, Ha SA, Park YG, Choi JY, Chung KW, Sun HS, Choi MJ, Chung J, Wands JR, Kim JW. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. *Cancer Res* 2004; **64**: 5434-5441 [PMID: 15289352 DOI: 10.1158/0008-5472.CAN-03-3665]

118 **Rasool M**, Rashid S, Arooj M, Ansari SA, Khan KM, Malik A, Naseer MI, Zahid S, Manan A, Asif M, Razzaq Z, Ashraf S, Qazi MH, Iqbal Z, Gan SH, Kamal MA, Sheikh IA. New possibilities in hepatocellular carcinoma treatment. *Anticancer Res* 2014; **34**: 1563-1571 [PMID: 24692683 DOI: 34/4/1563]

119 **Chiappini F**. Circulating tumor cells measurements in hepatocellular carcinoma. *Int J Hepatol* 2012; **2012**: 684802 [PMID: 22690340 DOI: 10.1155/2012/684802]

120 **Olaya N**, Chiappini F. Hepatocellular Carcinoma: Methods of Circulating Tumor Cells (CTC) Measurements. INTECH Open Access Publisher, 2012

121 **Reim M**, Grodau K, Kuhr M. Proceedings: Hexokinase activity and corneal nutrition. *Exp Eye Res* 1975; **20**: 179 [PMID: 1122968 DOI: 10.1016/S0140-6736(00)04046-0]

122 **Cescon M**, Bertuzzo VR, Ercolani G, Ravaioli M, Odaldi F, Pinna AD. Liver transplantation for hepatocellular carcinoma: role of inflammatory and immunological state on recurrence and prognosis. *World J Gastroenterol* 2013; **19**: 9174-9182 [PMID: 24409045 DOI: 10.3748/wjg.v19.i48.9174]

123 **Halazun KJ**, Hardy MA, Rana AA, Woodland DC, Luyten EJ, Mahadev S, Witkowski P, Siegel AB, Brown RS, Emond JC. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009; **250**: 141-151 [PMID: 19561458 DOI: 10.1097/SLA.0b013e3181a77e59]

124 **Walsh SR**, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol* 2005; **91**: 181-184 [PMID: 16118772 DOI: 10.1002/jso.20329]

125 **Halazun KJ**, Aldoori A, Malik HZ, Al-Mukhtar A, Prasad KR, Toogood GJ, Lodge JP. Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. *Eur J Surg Oncol* 2008; **34**: 55-60 [PMID: 17448623 DOI: 10.1016/j.ejso.2007.02.014]

126 **Tanigawa N**, Amaya H, Matsumura M, Shimomatsuya T. Correlation between expression of vascular endothelial growth factor and tumor vascularity, and patient outcome in human gastric carcinoma. *J Clin Oncol* 1997; **15**: 826-832 [PMID: 9053510]

127 **Kusumanto YH**, Dam WA, Hospers GA, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003; **6**: 283-287 [PMID: 15166496]

128 **Unitt E**, Marshall A, Gelson W, Rushbrook SM, Davies S, Vowler SL, Morris LS, Coleman N, Alexander GJ. Tumour lymphocytic infiltrate and recurrence of hepatocellular carcinoma following liver transplantation. *J Hepatol* 2006; **45**: 246-253 [PMID: 16580084 DOI: 10.1016/j.jhep.2005.12.027]

129 **Xiao GQ**, Liu C, Liu DL, Yang JY, Yan LN. Neutrophil-lymphocyte ratio predicts the prognosis of patients with hepatocellular carcinoma after liver transplantation. *World J Gastroenterol* 2013; **19**: 8398-8407 [PMID: 24363533 DOI: 10.3748/wjg.v19.i45.8398]

130 **Wang GY**, Yang Y, Li H, Zhang J, Jiang N, Li MR, Zhu HB, Zhang Q, Chen GH. A scoring model based on neutrophil to lymphocyte ratio predicts recurrence of HBV-associated hepatocellular carcinoma after liver transplantation. *PLoS One* 2011; **6**: e25295 [PMID: 21966488 DOI: 10.1371/journal.pone.0025295]

131 **Bertuzzo VR**, Cescon M, Ravaioli M, Grazi GL, Ercolani G, Del Gaudio M, Cucchetti A, D'Errico-Grigioni A, Golfieri R, Pinna AD. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. *Transplantation* 2011; **91**: 1279-1285 [PMID: 21617590 DOI: 10.1097/TP.0b013e3182187cf0]

132 **Limaye AR**, Clark V, Soldevila-Pico C, Morelli G, Suman A, Firpi R, Nelson DR, Cabrera R. Neutrophil-lymphocyte ratio predicts overall and recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Hepatol Res* 2013; **43**: 757-764 [PMID: 23193965 DOI: 10.1111/hepr.12019]

133 **Xiao WK**, Chen D, Li SQ, Fu SJ, Peng BG, Liang LJ. Prognostic significance of neutrophil-lymphocyte ratio in hepatocellular carcinoma: a meta-analysis. *BMC Cancer* 2014; **14**: 117 [PMID: 24559042 DOI: 10.1186/1471-2407-14-117]

134 **Parisi I**, Tsochatzis E, Wijewantha H, Rodríguez-Perálvarez M, De Luca L, Manousou P, Fatourou E, Pieri G, Papastergiou V, Davies N, Yu D, Luong T, Dhillon AP, Thorburn D, Patch D, O'Beirne J, Meyer T, Burroughs AK. Inflammation-based scores do not predict post-transplant recurrence of hepatocellular carcinoma in patients within Milan criteria. *Liver Transpl* 2014; **20**: 1327-1335 [PMID: 25088400 DOI: 10.1002/lt.23969]

135 **Lai Q**, Lerut J. Reply to 'neutrophil and platelet-to-lymphocyte ratio: new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer?'. *Transpl Int* 2014; **27**: e82-e83 [PMID: 24628991 DOI: 10.1111/tri.12191]

136 **Xia W**, Ke Q, Wang Y, Wang W, Zhang M, Shen Y, Wu J, Xu X, Zheng S. Predictive value of pre-transplant platelet to lymphocyte ratio for hepatocellular carcinoma recurrence after liver transplantation. *World J Surg Oncol* 2015; **13**: 60 [PMID: 25885777 DOI: 10.1186/s12957-015-0472-2]

137 **Argilés JM**, Busquets S, López-Soriano FJ. Cytokines in the pathogenesis of cancer cachexia. *Curr Opin Clin Nutr Metab Care* 2003; **6**: 401-406 [PMID: 12806213 DOI: 10.1097/01.mco.0000078983.18774.cc]

138 **Chan AW,** Chan SL, Wong GL, Wong VW, Chong CC, Lai PB, Chan HL, To KF. Prognostic Nutritional Index (PNI) Predicts Tumor Recurrence of Very Early/Early Stage Hepatocellular Carcinoma After Surgical Resection. *Ann Surg Oncol* 2015; Epub ahead of print [PMID: 25801356 DOI: 10.1245/s10434-015-4516-1]

139 **Toyoda H**, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Yamaguchi A, Isogai M, Kaneoka Y, Washizu J. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2006; **4**: 111-117 [PMID: 16431313 DOI: 10.1016/S1542-3565(05)00855-4]

140 **Fox R**, Berhane S, Teng M, Cox T, Tada T, Toyoda H, Kumada T, Kagebayashi C, Satomura S, Johnson PJ. Biomarker-based prognosis in hepatocellular carcinoma: validation and extension of the BALAD model. *Br J Cancer* 2014; **110**: 2090-2098 [PMID: 24691419 DOI: 10.1038/bjc.2014.130]

141 **Kiriyama S**, Uchiyama K, Ueno M, Ozawa S, Hayami S, Tani M, Yamaue H. Triple positive tumor markers for hepatocellular carcinoma are useful predictors of poor survival. *Ann Surg* 2011; **254**: 984-991 [PMID: 21606837 DOI: 10.1097/SLA.0b013e3182215016]

142 **Li X**, Yang W, Lou L, Chen Y, Wu S, Ding G. microRNA: a promising diagnostic biomarker and therapeutic target for hepatocellular carcinoma. *Dig Dis Sci* 2014; **59**: 1099-1107 [PMID: 24390674 DOI: 10.1007/s10620-013-3006-1]

143 **Criss WE**. A review of isozymes in cancer. *Cancer Res* 1971; **31**: 1523-1542 [PMID: 4399291]

144 **Khan MA**, Combs CS, Brunt EM, Lowe VJ, Wolverson MK, Solomon H, Collins BT, Di Bisceglie AM. Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. *J Hepatol* 2000; **32**: 792-797 [PMID: 10845666 DOI: 10.1016/S0168-8278(00)80248-2]

145 **Sugiyama M**, Sakahara H, Torizuka T, Kanno T, Nakamura F, Futatsubashi M, Nakamura S. 18F-FDG PET in the detection of extrahepatic metastases from hepatocellular carcinoma. *J Gastroenterol* 2004; **39**: 961-968 [PMID: 15549449 DOI: 10.1007/s00535-004-1427-5]

146 **Yang SH**, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, Yi NJ, Lee KU. The role of (18)F-FDG-PET imaging for the selection of liver transplantation candidates among hepatocellular carcinoma patients. *Liver Transpl* 2006; **12**: 1655-1660 [PMID: 16964589 DOI: 10.1002/lt.20861]

147 **Kornberg A**, Freesmeyer M, Bärthel E, Jandt K, Katenkamp K, Steenbeck J, Sappler A, Habrecht O, Gottschild D, Settmacher U. 18F-FDG-uptake of hepatocellular carcinoma on PET predicts microvascular tumor invasion in liver transplant patients. *Am J Transplant* 2009; **9**: 592-600 [PMID: 19191771 DOI: 10.1111/j.1600-6143.2008.02516.x]

148 **Kornberg A**, Küpper B, Tannapfel A, Büchler P, Krause B, Witt U, Gottschild D, Friess H. Patients with non-[18 F]fludeoxyglucose-avid advanced hepatocellular carcinoma on clinical staging may achieve long-term recurrence-free survival after liver transplantation. *Liver Transpl* 2012; **18**: 53-61 [PMID: 21850692 DOI: 10.1002/lt.22416]

149 **Lee SD**, Kim SH, Kim SK, Kim YK, Park SJ. Clinical Impact of 18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography in Living Donor Liver Transplantation for Advanced Hepatocellular Carcinoma. *Transplantation* 2015; **99**: 2142-2149 [PMID: 25905981 DOI: 10.1097/TP.0000000000000719]

150 **Torizuka T**, Tamaki N, Inokuma T, Magata Y, Sasayama S, Yonekura Y, Tanaka A, Yamaoka Y, Yamamoto K, Konishi J. In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. *J Nucl Med* 1995; **36**: 1811-1817 [PMID: 7562048]

151 **Ho CL**, Yu SC, Yeung DW. 11C-acetate PET imaging in hepatocellular carcinoma and other liver masses. *J Nucl Med* 2003; **44**: 213-221 [PMID: 12571212]

152 **Jadvar H**. Hepatocellular carcinoma and gastroenteropancreatic neuroendocrine tumors: potential role of other positron emission tomography radiotracers. *Semin Nucl Med* 2012; **42**: 247-254 [PMID: 22681673 DOI: 10.1053/j.semnuclmed.2012.02.001]

153 **Talbot JN**, Fartoux L, Balogova S, Nataf V, Kerrou K, Gutman F, Huchet V, Ancel D, Grange JD, Rosmorduc O. Detection of hepatocellular carcinoma with PET/CT: a prospective comparison of 18F-fluorocholine and 18F-FDG in patients with cirrhosis or chronic liver disease. *J Nucl Med* 2010; **51**: 1699-1706 [PMID: 20956466 DOI: 10.2967/jnumed.110.075507]

154 **Asman Y**, Evenson AR, Even-Sapir E, Shibolet O. [18F]fludeoxyglucose positron emission tomography and computed tomography as a prognostic tool before liver transplantation, resection, and loco-ablative therapies for hepatocellular carcinoma. *Liver Transpl* 2015; **21**: 572-580 [PMID: 25644857 DOI: 10.1002/lt.24083]

155 **Cheung TT**, Ho CL, Lo CM, Chen S, Chan SC, Chok KS, Fung JY, Yan Chan AC, Sharr W, Yau T, Poon RT, Fan ST. 11C-acetate and 18F-FDG PET/CT for clinical staging and selection of patients with hepatocellular carcinoma for liver transplantation on the basis of Milan criteria: surgeon's perspective. *J Nucl Med* 2013; **54**: 192-200 [PMID: 23321459 DOI: 10.2967/jnumed.112.107516]

156 **Wu HB**, Wang QS, Li BY, Li HS, Zhou WL, Wang QY. F-18 FDG in conjunction with 11C-choline PET/CT in the diagnosis of hepatocellular carcinoma. *Clin Nucl Med* 2011; **36**: 1092-1097 [PMID: 22064078 DOI: 10.1097/RLU.0b013e3182335df4]

157 **Buchbender C**, Heusner TA, Lauenstein TC, Bockisch A, Antoch G. Oncologic PET/MRI, part 1: tumors of the brain, head and neck, chest, abdomen, and pelvis. *J Nucl Med* 2012; **53**: 928-938 [PMID: 22582048 DOI: 10.2967/jnumed.112.105338]

158 **Lauwers GY**, Terris B, Balis UJ, Batts KP, Regimbeau JM, Chang Y, Graeme-Cook F, Yamabe H, Ikai I, Cleary KR, Fujita S, Flejou JF, Zukerberg LR, Nagorney DM, Belghiti J, Yamaoka Y, Vauthey JN. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. *Am J Surg Pathol* 2002; **26**: 25-34 [PMID: 11756766 DOI: 10.1097/00000478-200201000-00003]

159 **Yamanaka J**, Yamanaka N, Nakasho K, Tanaka T, Ando T, Yasui C, Kuroda N, Takata M, Maeda S, Matsushita K, Uematsu K, Okamoto E. Clinicopathologic analysis of stage II-III hepatocellular carcinoma showing early massive recurrence after liver resection. *J Gastroenterol Hepatol* 2000; **15**: 1192-1198 [PMID: 11106101 DOI: 10.1046/j.1440-1746.2000.02323.x]

160 **Esnaola NF**, Lauwers GY, Mirza NQ, Nagorney DM, Doherty D, Ikai I, Yamaoka Y, Regimbeau JM, Belghiti J, Curley SA, Ellis LM, Vauthey JN. Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation. *J Gastrointest Surg* 2002; **6**: 224-32; discussion 232 [PMID: 11992808 DOI: 10.1016/S1091-255X(01)00015-4]

161 **DuBay D**, Sandroussi C, Sandhu L, Cleary S, Guba M, Cattral MS, McGilvray I, Ghanekar A, Selzner M, Greig PD, Grant DR. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011; **253**: 166-172 [PMID: 21294289 DOI: 10.1097/SLA.0b013e31820508f1]

162 **Pawlik TM**, Gleisner AL, Anders RA, Assumpcao L, Maley W, Choti MA. Preoperative assessment of hepatocellular carcinoma tumor grade using needle biopsy: implications for transplant eligibility. *Ann Surg* 2007; **245**: 435-442 [PMID: 17435551 DOI: 10.1097/01.sla.0000250420.73854.ad]

163 **Kenmochi K**, Sugihara S, Kojiro M. Relationship of histologic grade of hepatocellular carcinoma (HCC) to tumor size, and demonstration of tumor cells of multiple different grades in single small HCC. *Liver* 1987; **7**: 18-26 [PMID: 3033422 DOI: 10.1111/j.1600-0676.1987.tb00310.x]

164 **Sugihara S**, Nakashima O, Kojiro M, Majima Y, Tanaka M, Tanikawa K. The morphologic transition in hepatocellular carcinoma. A comparison of the individual histologic features disclosed by ultrasound-guided fine-needle biopsy with those of autopsy. *Cancer* 1992; **70**: 1488-1492 [PMID: 1325272 DOI: 3.0.CO; 2-J']

165 **EDMONDSON HA**, STEINER PE. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer* 1954; **7**: 462-503 [PMID: 13160935 DOI: 3.0.CO; 2-E']

166 **Huang P,** Lai Y. Effective segmentation and classification for HCC biopsy images. *Patt Recogt* 2010; **43**: 1550-1563 [DOI: 10.1016/j.patcog.2009.10.014]

167 **Kulesza P**, Torbenson M, Sheth S, Erozan YS, Ali SZ. Cytopathologic grading of hepatocellular carcinoma on fine-needle aspiration. *Cancer* 2004; **102**: 247-258 [PMID: 15368317 DOI: 10.1002/cncr.20409]

168 **Myers RP**, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008; **28**: 705-712 [PMID: 18433397 DOI: 10.1111/j.1478-3231.2008.01691.x]

169 **Durand F**, Belghiti J, Paradis V. Liver transplantation for hepatocellular carcinoma: role of biopsy. *Liver Transpl* 2007; **13**: S17-S23 [PMID: 17969095 DOI: 10.1002/lt.21326]

170 **Yu SC**, Lo DY, Ip CB, Liew CT, Leung TW, Lau WY. Does percutaneous liver biopsy of hepatocellular carcinoma cause hematogenous dissemination? An in vivo study with quantitative assay of circulating tumor DNA using methylation-specific real-time polymerase chain reaction. *AJR Am J Roentgenol* 2004; **183**: 383-385 [PMID: 15269029 DOI: 10.2214/ajr.183.2.1830383]

171 **Colecchia A**, Scaioli E, Montrone L, Vestito A, Di Biase AR, Pieri M, D'Errico-Grigioni A, Bacchi-Reggiani ML, Ravaioli M, Grazi GL, Festi D. Pre-operative liver biopsy in cirrhotic patients with early hepatocellular carcinoma represents a safe and accurate diagnostic tool for tumour grading assessment. *J Hepatol* 2011; **54**: 300-305 [PMID: 21056498 DOI: 10.1016/j.jhep.2010.06.037]

172 **Streba LAM,** Streba CT, Georgescu EF. Risks and Benefits of Liver Biopsy in Focal Liver Disease. INTECH Open Access Publisher, 2012 [DOI: 10.5772/52620]

173 **Denzer U**, Arnoldy A, Kanzler S, Galle PR, Dienes HP, Lohse AW. Prospective randomized comparison of minilaparoscopy and percutaneous liver biopsy: diagnosis of cirrhosis and complications. *J Clin Gastroenterol* 2007; **41**: 103-110 [PMID: 17198072 DOI: 10.1097/01.mcg.0000225612.86846.82]

174 **Helmreich-Becker I**, Meyer zum Büschenfelde KH, Lohse AW. Safety and feasibility of a new minimally invasive diagnostic laparoscopy technique. *Endoscopy* 1998; **30**: 756-762 [PMID: 9932754 DOI: 10.1055/s-2007-1001417]

175 **European Association For The Study Of The Liver;** European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

176 **DeRisi J**, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 1996; **14**: 457-460 [PMID: 8944026 DOI: 10.1038/ng1296-457]

177 **Pinyol R**, Nault JC, Quetglas IM, Zucman-Rossi J, Llovet JM. Molecular profiling of liver tumors: classification and clinical translation for decision making. *Semin Liver Dis* 2014; **34**: 363-375 [PMID: 25369299 DOI: 10.1055/s-0034-1394137]

178 **Ho MC**, Lin JJ, Chen CN, Chen CC, Lee H, Yang CY, Ni YH, Chang KJ, Hsu HC, Hsieh FJ, Lee PH. A gene expression profile for vascular invasion can predict the recurrence after resection of hepatocellular carcinoma: a microarray approach. *Ann Surg Oncol* 2006; **13**: 1474-1484 [PMID: 17009164 DOI: 10.1245/s10434-006-9057-1]

179 **Ye QH**, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, Li Y, Robles AI, Chen Y, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med* 2003; **9**: 416-423 [PMID: 12640447 DOI: 10.1038/nm843]

180 **Chen X**, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D, Brown PO. Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002; **13**: 1929-1939 [PMID: 12058060 DOI: 10.1091/mbc.02-02-0023.]

181 **Villa E,** Critelli R, Lei B, Marzocchi G, Camma C, Giannelli G, Pontisso P, Cabibbo G, Enea M, Colopi S, Caporali C, Pollicino T, Milosa F, Karampatou A, Todesca P, Bertolini E, Maccio L, Martinez-Chantar ML, Turola E, Del Buono M, De Maria N, Ballestri S, Schepis F, Loria P, Enrico Gerunda G, Losi L, Cillo U. Neoangiogenesis-related genes are hallmarks of fast-growing hepatocellular carcinomas and worst survival. Results from a prospective study. *Gut* 2015; Epub ahead of print [PMID: 25666192 DOI: gutjnl-2014-308483]

182 **Iizuka N**, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, Tamesa T, Tangoku A, Tabuchi H, Hamada K, Nakayama H, Ishitsuka H, Miyamoto T, Hirabayashi A, Uchimura S, Hamamoto Y. Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. *Lancet* 2003; **361**: 923-929 [PMID: 12648972]

183 **Lee JS**, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgeirsson SS. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004; **40**: 667-676 [PMID: 15349906 DOI: 10.1002/hep.20375]

184 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 1995-2004 [PMID: 18923165 DOI: 10.1056/NEJMoa0804525]

185 **Huang YS**, Dai Y, Yu XF, Bao SY, Yin YB, Tang M, Hu CX. Microarray analysis of microRNA expression in hepatocellular carcinoma and non-tumorous tissues without viral hepatitis. *J Gastroenterol Hepatol* 2008; **23**: 87-94 [PMID: 18171346 DOI: 10.1111/j.1440-1746.2007.05223.x]

186 **Mah WC**, Lee CG. DNA methylation: potential biomarker in Hepatocellular Carcinoma. *Biomark Res* 2014; **2**: 5 [PMID: 24635883 DOI: 10.1186/2050-7771-2-5]

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

188 **Majno PE**, Adam R, Bismuth H, Castaing D, Ariche A, Krissat J, Perrin H, Azoulay D. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. *Ann Surg* 1997; **226**: 688-701; discussion 701-3 [PMID: 9409568 DOI: 10.1097/00000658-199712000-00006]

189 **Herrero JI**, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, Prieto J. Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma. *Liver Transpl* 2001; **7**: 631-636 [PMID: 11460231 DOI: 10.1053/jlts.2001.25458]

190 **Graziadei IW**, Sandmueller H, Waldenberger P, Koenigsrainer A, Nachbaur K, Jaschke W, Margreiter R, Vogel W. Chemoembolization followed by liver transplantation for hepatocellular carcinoma impedes tumor progression while on the waiting list and leads to excellent outcome. *Liver Transpl* 2003; **9**: 557-563 [PMID: 12783395 DOI: 10.1053/jlts.2003.50106]

191 **Yao FY**, Bass NM, Nikolai B, Merriman R, Davern TJ, Kerlan R, Ascher NL, Roberts JP. A follow-up analysis of the pattern and predictors of dropout from the waiting list for liver transplantation in patients with hepatocellular carcinoma: implications for the current organ allocation policy. *Liver Transpl* 2003; **9**: 684-692 [PMID: 12827553 DOI: 10.1053/jlts.2003.50147]

192 **Hayashi PH**, Trotter JF, Forman L, Kugelmas M, Steinberg T, Russ P, Wachs M, Bak T, Kam I, Everson GT. Impact of pretransplant diagnosis of hepatocellular carcinoma on cadveric liver allocation in the era of MELD. *Liver Transpl* 2004; **10**: 42-48 [PMID: 14755776 DOI: 10.1002/lt.20020]

193 **Fisher RA**, Maluf D, Cotterell AH, Stravitz T, Wolfe L, Luketic V, Sterling R, Shiffman M, Posner M. Non-resective ablation therapy for hepatocellular carcinoma: effectiveness measured by intention-to-treat and dropout from liver transplant waiting list. *Clin Transplant* 2004; **18**: 502-512 [PMID: 15344951 DOI: 10.1111/j.1399-0012.2004.00196.x]

194 **Maddala YK**, Stadheim L, Andrews JC, Burgart LJ, Rosen CB, Kremers WK, Gores G. Drop-out rates of patients with hepatocellular cancer listed for liver transplantation: outcome with chemoembolization. *Liver Transpl* 2004; **10**: 449-455 [PMID: 15004776 DOI: 10.1002/lt.20099]

195 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556 DOI: 10.1002/lt.20099]

196 **Porrett PM**, Peterman H, Rosen M, Sonnad S, Soulen M, Markmann JF, Shaked A, Furth E, Reddy KR, Olthoff K. Lack of benefit of pre-transplant locoregional hepatic therapy for hepatocellular cancer in the current MELD era. *Liver Transpl* 2006; **12**: 665-673 [PMID: 16482577 DOI: 10.1002/lt.20636]

197 **Huo TI**, Lin HC, Huo SC, Lee PC, Wu JC, Lee FY, Hou MC, Lee SD. Comparison of four model for end-stage liver disease-based prognostic systems for cirrhosis. *Liver Transpl* 2008; **14**: 837-844 [PMID: 18508377 DOI: 10.1002/lt.21439]

198 **De Giorgio M**, Vezzoli S, Cohen E, Armellini E, Lucà MG, Verga G, Pinelli D, Nani R, Valsecchi MG, Antolini L, Colledan M, Fagiuoli S, Strazzabosco M. Prediction of progression-free survival in patients presenting with hepatocellular carcinoma within the Milan criteria. *Liver Transpl* 2010; **16**: 503-512 [PMID: 20373461 DOI: 10.1002/lt.22039]

199 **Cucchetti A**, Cescon M, Bigonzi E, Piscaglia F, Golfieri R, Ercolani G, Cristina Morelli M, Ravaioli M, Daniele Pinna A. Priority of candidates with hepatocellular carcinoma awaiting liver transplantation can be reduced after successful bridge therapy. *Liver Transpl* 2011; **17**: 1344-1354 [PMID: 21837731 DOI: 10.1002/lt.22397]

200 **Ciccarelli O**, Lai Q, Goffette P, Finet P, De Reyck C, Roggen F, Sempoux C, Doffagne E, Reding R, Lerut J. Liver transplantation for hepatocellular cancer: UCL experience in 137 adult cirrhotic patients. Alpha-foetoprotein level and locoregional treatment as refined selection criteria. *Transpl Int* 2012; **25**: 867-875 [PMID: 22716073 DOI: 10.1111/j.1432-2277.2012.01512.x]

201 **Decaens T**, Roudot-Thoraval F, Bresson-Hadni S, Meyer C, Gugenheim J, Durand F, Bernard PH, Boillot O, Boudjema K, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Dharancy S, Chazouilleres O, Dhumeaux D, Cherqui D, Duvoux C. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2005; **11**: 767-775 [PMID: 15973710 DOI: 10.1002/lt.20418]

202 **Pelletier SJ**, Fu S, Thyagarajan V, Romero-Marrero C, Batheja MJ, Punch JD, Magee JC, Lok AS, Fontana RJ, Marrero JA. An intention-to-treat analysis of liver transplantation for hepatocellular carcinoma using organ procurement transplant network data. *Liver Transpl* 2009; **15**: 859-868 [PMID: 19642139 DOI: 10.1002/lt.21778]

203 **Lesurtel M**, Müllhaupt B, Pestalozzi BC, Pfammatter T, Clavien PA. Transarterial chemoembolization as a bridge to liver transplantation for hepatocellular carcinoma: an evidence-based analysis. *Am J Transplant* 2006; **6**: 2644-2650 [PMID: 16939518 DOI: 10.1111/j.1600-6143.2006.01509.x]

204 **Majno P**, Giostra E, Mentha G. Management of hepatocellular carcinoma on the waiting list before liver transplantation: time for controlled trials? *Liver Transpl* 2007; **13**: S27-S35 [PMID: 17969086 DOI: 10.1002/lt.21328]

205 **Llovet JM**, Mas X, Aponte JJ, Fuster J, Navasa M, Christensen E, Rodés J, Bruix J. Cost effectiveness of adjuvant therapy for hepatocellular carcinoma during the waiting list for liver transplantation. *Gut* 2002; **50**: 123-128 [PMID: 11772979 DOI: 10.1136/gut.50.1.123]

206 **Roayaie S**, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797 DOI: 10.1002/lt.20128]

207 **Otto G**, Schuchmann M, Hoppe-Lotichius M, Heise M, Weinmann A, Hansen T, Pitton MP. How to decide about liver transplantation in patients with hepatocellular carcinoma: size and number of lesions or response to TACE? *J Hepatol* 2013; **59**: 279-284 [PMID: 23587474 DOI: 10.1016/j.jhep.2013.04.006]

208 **Allard MA**, Sebagh M, Ruiz A, Guettier C, Paule B, Vibert E, Cunha AS, Cherqui D, Samuel D, Bismuth H, Castaing D, Adam R. Does pathological response after transarterial chemoembolization for hepatocellular carcinoma in cirrhotic patients with cirrhosis predict outcome after liver resection or transplantation? *J Hepatol* 2015; **63**: 83-92 [PMID: 25646884 DOI: 10.1016/j.jhep.2015.01.023]

209 **Ho MH**, Yu CY, Chung KP, Chen TW, Chu HC, Lin CK, Hsieh CB. Locoregional therapy-induced tumor necrosis as a predictor of recurrence after liver transplant in patients with hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 3632-3639 [PMID: 21626078 DOI: 10.1245/s10434-011-1803-3]

210 **Roberts JP**, Venook A, Kerlan R, Yao F. Hepatocellular carcinoma: Ablate and wait versus rapid transplantation. *Liver Transpl* 2010; **16**: 925-929 [PMID: 20658555 DOI: 10.1002/lt.22103]

211 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J. 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**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]

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

213 **De Luna W**, Sze DY, Ahmed A, Ha BY, Ayoub W, Keeffe EB, Cooper A, Esquivel C, Nguyen MH. Transarterial chemoinfusion for hepatocellular carcinoma as downstaging therapy and a bridge toward liver transplantation. *Am J Transplant* 2009; **9**: 1158-1168 [PMID: 19344435 DOI: 10.1111/j.1600-6143.2009.02576.x]

214 **Yao FY**, Mehta N, Flemming J, Dodge J, Hameed B, Fix O, Hirose R, Fidelman N, Kerlan RK, Roberts JP. Downstaging of hepatocellular cancer before liver transplant: long-term outcome compared to tumors within Milan criteria. *Hepatology* 2015; **61**: 1968-1977 [PMID: 25689978 DOI: 10.1002/hep.27752]

215 **Park JO**, Lee SI, Song SY, Kim K, Kim WS, Jung CW, Park YS, Im YH, Kang WK, Lee MH, Lee KS, Park K. Measuring response in solid tumors: comparison of RECIST and WHO response criteria. *Jpn J Clin Oncol* 2003; **33**: 533-537 [PMID: 14623923 DOI: 10.1093/jjco/hyg093]

216 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]

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

218 **Llovet JM**, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 698-711 [PMID: 18477802 DOI: 10.1093/jnci/djn134]

219 **Therasse P**, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205-216 [PMID: 10655437 DOI: 10.1093/jnci/92.3.205]

220 **Raoul J**, Park J, Kang Y, Finn R, Kim J, Yeo W, Polite B, Chao Y, Walters I, Baudelet C. Using Modified RECIST and Alpha-Fetoprotein Levels to Assess Treatment Benefit in Hepatocellular Carcinoma. *Liver Cancer* 2014; **3**: 439-450 [DOI: 10.1159/000343872]

221 **Kojiro M**, Sugihara S, Kakizoe S, Nakashima O, Kiyomatsu K. Hepatocellular carcinoma with sarcomatous change: a special reference to the relationship with anticancer therapy. *Cancer Chemother Pharmacol* 1989; **23 Suppl**: S4-S8 [PMID: 2466583 DOI: 10.1007/BF00647229]

222 **Koda M**, Maeda Y, Matsunaga Y, Mimura K, Murawaki Y, Horie Y. Hepatocellular carcinoma with sarcomatous change arising after radiofrequency ablation for well-differentiated hepatocellular carcinoma. *Hepatol Res* 2003; **27**: 163-167 [PMID: 14563432 DOI: 10.1016/S1386-6346(03)00207-9]

223 **Takada Y**, Kurata M, Ohkohchi N. Rapid and aggressive recurrence accompanied by portal tumor thrombus after radiofrequency ablation for hepatocellular carcinoma. *Int J Clin Oncol* 2003; **8**: 332-335 [PMID: 14586761 DOI: 10.1007/s10147-003-0328-6]

224 **Zen C**, Zen Y, Mitry RR, Corbeil D, Karbanová J, O'Grady J, Karani J, Kane P, Heaton N, Portmann BC, Quaglia A. Mixed phenotype hepatocellular carcinoma after transarterial chemoembolization and liver transplantation. *Liver Transpl* 2011; **17**: 943-954 [PMID: 21491582 DOI: 10.1002/lt.22314]

225 **Yamamoto N**, Okano K, Kushida Y, Deguchi A, Yachida S, Suzuki Y. Clinicopathology of recurrent hepatocellular carcinomas after radiofrequency ablation treated with salvage surgery. *Hepatol Res* 2014; **44**: 1062-1071 [PMID: 23957810 DOI: 10.1111/hepr.12223]

226 **Kong J**, Kong L, Kong J, Ke S, Gao J, Ding X, Zheng L, Sun H, Sun W. After insufficient radiofrequency ablation, tumor-associated endothelial cells exhibit enhanced angiogenesis and promote invasiveness of residual hepatocellular carcinoma. *J Transl Med* 2012; **10**: 230 [PMID: 23171368 DOI: 10.1186/1479-5876-10-230]

227 **Toso C**, Mentha G, Kneteman NM, Majno P. The place of downstaging for hepatocellular carcinoma. *J Hepatol* 2010; **52**: 930-936 [PMID: 20385428 DOI: 10.1016/j.jhep.2009.12.032]

228 **SCHWARTZ M**. A biomathematical approach to clinical tumor growth. *Cancer* 1961; **14**: 1272-1294 [PMID: 13909709 DOI: 3.0.CO; 2-H']

229 **Nakajima T**, Moriguchi M, Mitsumoto Y, Katagishi T, Kimura H, Shintani H, Deguchi T, Okanoue T, Kagawa K, Ashihara T. Simple tumor profile chart based on cell kinetic parameters and histologic grade is useful for estimating the natural growth rate of hepatocellular carcinoma. *Hum Pathol* 2002; **33**: 92-99 [PMID: 11823978 DOI: 10.1053/hupa.2002.30194]

230 **Okazaki N**, Yoshino M, Yoshida T, Suzuki M, Moriyama N, Takayasu K, Makuuchi M, Yamazaki S, Hasegawa H, Noguchi M. Evaluation of the prognosis for small hepatocellular carcinoma based on tumor volume doubling time. A preliminary report. *Cancer* 1989; **63**: 2207-2210 [PMID: 2541886 DOI: 3.0.CO; 2-C']

231 **Barbara L**, Benzi G, Gaiani S, Fusconi F, Zironi G, Siringo S, Rigamonti A, Barbara C, Grigioni W, Mazziotti A. Natural history of small untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumor growth rate and patient survival. *Hepatology* 1992; **16**: 132-137 [PMID: 1352268 DOI: 10.1002/hep.1840160122]

232 **Cucchetti A**, Vivarelli M, Piscaglia F, Nardo B, Montalti R, Grazi GL, Ravaioli M, La Barba G, Cavallari A, Bolondi L, Pinna AD. Tumor doubling time predicts recurrence after surgery and describes the histological pattern of hepatocellular carcinoma on cirrhosis. *J Hepatol* 2005; **43**: 310-316 [PMID: 15970351 DOI: 10.1016/j.jhep.2005.03.014]

233 **Kubota K**, Ina H, Okada Y, Irie T. Growth rate of primary single hepatocellular carcinoma: determining optimal screening interval with contrast enhanced computed tomography. *Dig Dis Sci* 2003; **48**: 581-586 [PMID: 12757173 DOI: 10.1023/A: 1022505203786]

234 **Sheu JC**, Sung JL, Chen DS, Yang PM, Lai MY, Lee CS, Hsu HC, Chuang CN, Yang PC, Wang TH. Growth rate of asymptomatic hepatocellular carcinoma and its clinical implications. *Gastroenterology* 1985; **89**: 259-266 [PMID: 2408960 DOI: S0016508585002220]

235 **Ebara M**, Ohto M, Shinagawa T, Sugiura N, Kimura K, Matsutani S, Morita M, Saisho H, Tsuchiya Y, Okuda K. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis. A study in 22 patients. *Gastroenterology* 1986; **90**: 289-298 [PMID: 2416627]

236 **Yoshino M**. Growth kinetics of hepatocellular carcinoma. *Jpn J Clin Oncol* 1983; **13**: 45-52 [PMID: 6187947]

237 **Woo HY**, Jang JW, Choi JY, Bae SH, You CR, Rha SE, Lee YJ, Yoon SK, Lee CD. Tumor doubling time after initial response to transarterial chemoembolization in patients with hepatocellular carcinoma. *Scand J Gastroenterol* 2010; **45**: 332-339 [PMID: 20001605 DOI: 10.3109/00365520903456573]

**P-Reviewer:** Dirchwolf M, Vilaichone RK **S-Editor:** Qi Y **L-Editor: E-Editor:**

**Table 1 Hepatocellular carcinoma patients’ pre-transplant α-fetoprotein level, outcome, and biological features according to some recent studies**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **No. of patients** | **Static AFP cut-off (μg/L)** | **Dynamic AFP**  | **Outcome for increasing AFP ranges** | ***P* value** | **Other biological features** |
| Berry *et al*[31] (2013) | 8659 | ≤ 15, 16-66, 66-320, > 320 | - | 6 yr OS: from 70% to 60%, 57%, 51%  | - | - |
| Toso *et al*[26] (2011) | 6478 | ≤ 100, 100-500, > 500 | - | 3 yr OS: from 71% to 60%, 51%  | < 0.001 | - |
| Mailey *et al*[32] (2011) | 2253 | ≤ 20, > 400 | - | 4 yr OS: from 76% to 54% | < 0.001 | - |
| Duvoux *et al*[20] (2012) | 1033 | ≤ 100, 100-1000, > 1000 | - | 5 yr OS: from 68% to 51%, 39% | < 0.001  | VI, poor differentiation |
| Todo *et al*[30] (2007) | 653 | ≤ 200, > 1000 | - | 5 yr OS: from 73% to 34% | < 0.001 | - |
| Fujiki *et al*[41] (2009) | 144 | ≤ 200, > 800 | - |  5 yr RFS: from 90% to 40% | 0.003 | VI, poor differentiation |
| Sotiropolous *et al*[12] | 100 | ≤ 20, 20-200, 200-1000, > 1000 | - |  5 yr RFS: from 97% to 60%, 57%, 51%  | 0.0003 | - |
| Hameed *et al*[45] | 211 | ≤ 1000, > 1000 | - | 5- yr RFS: from 80.3% to 52.7% | 0.025 | VI |
| Kondili *et al*[46] | 32 | - | grate increasing, low increasing |  In 5 Patents with recurrence AFP increased at a greater magnitude than in 27 without recurrence | - | - |
| Han *et al*[28] | 48 | - | ≤ 50 μg/L per month, > 50 μg/L per month | 1 yr RFS: from 90% to 40% | < 0.001 | VI |
| Vibert *et al*[29] | 153 | - | ≤ 15 μg/L per month, > 15 μg/L per month | 5 yr RFS: from 76% to 54% | 0.01 | VI |
| Merani *et al*[47] | 6817 | - | Stable, ≥ 400, downstaged to < 400 | ITT survival: from 81% to 48% | < .001 | - |

OS: Overall survival rate; RFS: Recurrence-free survival rate; ITT: Intention-to-treat; VI: Vascular invasion.

**Table 2 Response to therapy: comparison of outcomes following different pre-transplant strategies**

|  |
| --- |
|  |
| **Author** | **Treatment** | **Response assessemnt** | **Transplant criteria** | **N patients** | **Outcome** | **Comparison between Responders and Non Responders** | **Comparison between downstaged patient *vs* conventional criteria** |
| Millonig *et al*[169]  | TACE | RECIST | UCSF | *total* | 116 | *total* | - | 　 | 　 |
| *downstaging (DS)* | NA | *DS responders*  | 5-yr OS = 25% | 　 | NS1 |
| *bridging (B)* | NA | *B responders*  | 5-yr OS = 85.7% | 0.02 |
| *B non responders* | 5-yr OS = 51.4% |
| Chapman *et al*[193] | Resection, ablation, TACE | RECIST | Milan | *total* | 136 | *total* | - | 　 | 　 |
| *downstaging (DS)* | 76 | *DS responders*  | 5-yr RFS = 50% | 　 | NS |
| *bridging (B)* | 60 | *B responders*  | 5-y RFS = 62.6% | NA |
| *B non responders* |
| Vitale *et al*[15] | Resection, ablation, TACE | RECIST | Milan | *total* | 147 |  | 5-yr ITT survival = 74% | 　 | 　 |
| *downstaging (DS)* | NA | *DS responders*  | - | 　 | NA |
| *bridging (B)* | NA | *B responders*  | 5-yr ITT survival = 83% | < 0.01 |
| *B non responders* | 5-yr ITT survival = 63% |
| Cucchetti *et al*[181] | Resection, ablation, TACE | mRECIST | Milan | *total* | 315 | *total* | - | 　 | 　 |
| *downstaging (DS)* | 53 | *DS responders*  | 5-yr RR = 19.2% | 　 | NS1 |
| *bridging (B)* | 240 | *B responders*  | 5-yr RR = 5.5% | 0.017 |
| *B non responders* | 5-yr RR = 19.4% |
| Ravaioli *et al*[17] | Resection, ablation, TACE | RECIST | Milan | *total* | 177 | *total* | 3-yr RFS = 82% | 　 | 　 |
| *downstaging (DS)* | 48 | *DS responders*  | 3-yr RFS = 75% | 　 | NS |
| *bridging (B)* | NA | *B responders*  | 3-yr RFS = 83% | NA |
| *B non responders* |
| Yao *et al*[195] | Resection, ablation, TACE | mRECIST | Milan | *total* | 606 | *total* | - | 　 | 　 |
| *downstaging (DS)* | 118 | *DS responders*  | 5-yr RFS = 90.8% | 　 | NS |
| *bridging (B)* | NA | *B responders*  | 5-yr RFS = 88% | NA |
| *B non responders* |
| De Luna *et al*[194] | TACI | NA | Milan | *total* | 122 | *total* | 3-yr OS = 82.7% | 　 | 　 |
| *downstaging (DS)* | 27 | *DS responders*  | 3-yr OS = 84.1% | 　 | NS |
| *bridging (B)* | NA | *B responders*  | 3-yr OS = 84.7% | NA |
| *B non responders* |
| Graziadei *et al*[172] | TACE | NA | Milan | *total* | 63 | *total* | NA | 　 | 　 |
| *downstaging (DS)* | 15 | *DS responders*  | 4-yr OS = 41% | 　 | NA |
| *bridging (B)* | 48 | *B responders*  | 5-yr OS = 94% | NA |
| *B non responders* | na |
| Otto *et al*[189] | TACE | mRECIST | Milan | *total* | 136 | *total* | - | 　 | 　 |
| *downstaging (DS)* | 49 | *DS responders*  | 5-yr RFS = 92% | < 0.0001 | NA |
| *bridging (B)* | 87 | *B responders*  |
| *B non responders* | 5-yr RFS = 22% |
| De Giorgio *et al*[180] | Resection, ablation, TACE | NA | Milan | *total* | 206 | *total* | NA | 　 | 　 |
| *downstaging (DS)* | NA | *DS responders*  | 　 | NA |
| *bridging (B)* | 83 | *B responders*  | NA |
| *B non responders* |
|  |

1Computed not stastically significant. NA: Not available; NS: Not statistically significant; OS: Overall Survival; RFS: Recurrence free survival or freedom from recurrence; RR: Recurrence rate; RECIST: Response Evaluation criteria in solid tumors; mRECIST: Modified response evaluation criteria in solid tumors; TACE: Trans arterial chemo-embolization; TACI: Trans Arterial chemo-infusion; UCSF: University of California San Francisco.

**Table 3 Integrated selection criteria schemes for liver transplantation in hepatocellular carcinoma patients**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Author** |  **Model** | **No. of Patients** | **Parameters** | **Cut-off (points)** | **Criteria** | **Endpoint** | **Criteria-in outcome** | **Criteria-out outcome** | **Validation** |
| Toso *et al*[26]  | TTV/AFP | 6478 | TTV | ≤ 115 cm³, > 115 cm³ | TTV ≤ 115 cm³ AND AFP ≤ 400 ng/m | Corrected posttransplant 3-year OS  | > 65% | < 50% | Grat *et al*: 104 patients with similar results |
| AFP | ≤ 400 ng/mL, > 400 ng/mL |
| Douvoux *et al*[20] | The AFP Model | Training cohort: 597 Validation cohort: 435 | Longest Diameter | < 3 cm (0), 3-6 cm (1), > 6 cm (4) | Sum of individual points ≤ 2 | posttransplant 5-year RFS | 7.7 % (Milan-in) 14.4 % (Milan-out) | 53.3 % (Milan-in) 47.6 % (Milan-out) | Notarpaolo *et al*[44]: 560 patients with similar results |
| No. of nodules | 1-3 (0), ≥ 4 (2) |
| AFP | < 100 ng/mL (0), 100-1000 (2), > 1000 (3) |
| Lai *et al*[192] | - | 422 | mRECIST | Progression *vs* No progression | No progression AND AFP solpe ≤ 15 | 5-year RFS 5-year OS | RFS: 90% (Milan-in), 87% (Milan-out) OS: 88% (Milan-in), 83.5% (Milan-out) | RFS: 67.7% (Milan-in) 47% (Milan-out) OS 67.3% (Milan-in) 55.4% (Milan-out) | Not yet validated |
| AFP slope | ≤ 15 ng/mL per mo, > 15 ng/mL per mo |

AFP: Alpha-fetoprotein.