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Review article

Usefulness of pre-surgical biopsy in selecting patients with hepatocellular carcinoma for liver transplant

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ABSTRACT

The selection of patients with hepatocellular carcinoma (HCC) for liver transplantation must be improved. One of the methods proposed to achieve this objective consists of including predictors of tumour aggressiveness to the decision making algorithm. The procedures that would enable this characteristic to be assessed, are: 1. Serum biomarkers, 2. Response to transarterial chemoembolisation and 3. Data on the tumour histology. In this review, the available data on the usefulness of each of these procedures are analysed. Special attention is given to the evidence associated with the possible usefulness of a preoperative biopsy. It can be concluded that a preoperative biopsy could be useful to indicate liver transplantation in patients with extended criteria, but not in patients that fulfil the Milan criteria. This scenario could soon change if the initial data on the prognostic value of some molecular markers of tumour progression are confirmed.

Utilidad de la biopsia preoperatoria en la selección de pacientes con hepatocarcinoma para el trasplante hepático

Palabras clave:
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RESUMEN

La selección de pacientes con hepatocarcinoma para el trasplante hepático es susceptible de mejorarse. Uno de los métodos propuestos para lograr este objetivo consiste en la incorporación al algoritmo decisorio de factores pronóstico de agresividad tumoral. Los procedimientos que permitirían valorar esta característica pueden agruparse en 3 categorías: *a)* biomarcadores séricos; *b)* respuesta a la quimioembolización, y *c)* datos de la histología tumoral. En este estudio de revisión se analizan los datos disponibles acerca de la utilidad de cada uno de estos tipos de marcadores, y se presta una especial atención a las evidencias relacionadas con la posible utilidad de una biopsia preoperatoria. Puede concluirse que la biopsia preoperatoria podría ser útil para indicar el trasplante hepático en pacientes con criterios expandidos, pero no en pacientes que cumplan los criterios de Milán. Este escenario podría cambiar en un futuro no muy lejano si se confirman los primeros datos del valor pronóstico de algunos parámetros de biología molecular.

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Selection of patients with hepatocellular carcinomas (HCC) for liver transplantation (LT) is based on morphological criteria established by Mazzaferro in 1996.[1] Without a doubt, the results obtained from the application of these criteria are excellent. However, the limitations of imaging techniques create a situation in which the final tumour stage exceeds the accepted criteria in 20% to 30% of cases. It is precisely these limitations of the preoperative staging that have highlighted the existence of a group of patients with excellent LT survival rates in spite of exceeding Milan criteria.

These observations emphasize that the selection of HCC patients for LT can be improved, both through improvement of imaging technique precision and, more importantly, by detection of prognostic factors for tumour aggressiveness.

The objective of this literature review study was to analyse the available information regarding the different procedures applied in evaluating tumour aggressiveness. These procedures can be grouped into 3 categories: *a)* serum biomarkers; *b)* response to chemoembolization (CHE), along with an observation period, and *c)* tumour histology.

Serum biomarkers

HCCs are characterized by highly vascularised tumours with a strong propensity for venous invasions. As a result, there is a possible relationship between the presence of vascular endothelial growth factor (VEGF) and the aggressiveness of the HCC. In a prospective study by Poon et al,[2] preoperative VEGF levels were correlated with clinical pathological data from 100 consecutive patients treated with curative hepatectomies.

The statistical analysis showed that with a cut-off at the 75th percentile (500 pg/ml), an elevated level of this molecule is associated with greater risk of recurrence (48 as opposed to 27%) following a mean 12-month observation period (P=.048). Illness-free survival was lower in the group with elevated levels, but these differences were not significant. Patients with levels of VEGF > 500 pg/ml had a 48% incidence of microscopic vascular invasion compared to 27%.

Following this publication, another 300 articles can be found that study the relationship between VEGF and HCCs. However, no studies exist that investigate its usefulness as a prognostic factor in transplant patients.

Des-gamma-carboxy prothrombin is an abnormal form of prothrombin, known for years as a serum marker for HCC. In a study by Shirabe et al[3] in 2007, levels higher than 500mAU/ml correlated with a vascular invasion in 54% of cases, apparently insufficient for making decisions based on this information alone.

Detection of cells with alphaprotein mRNA expression in peripheral blood has been studied as a factor for predicting recurrence. Intuitively, these results seem to indicate a high risk of haematogenous spread. In the multivariate analysis performed by Marubashi et al[4] with 32 patients transplanted for HCC, vascular invasion and the presence of cells expressing alphaprotein mRNA turned out to be prognostic factors for recurrence, while size and Milan criteria compliance were insignificant. Furthermore, among those patients that surpassed the Milan criteria, the mRNA study produced 2 groups of patients with very different prognoses. However, the number of patients with mRNA expression in the blood during the preoperative study was only 3 cases, and all of these had HCCs that exceeded the Milan criteria. As a result, this parameter seems not to offer much clinical usefulness either.

In summary there appears to be no solid evidence that would permit recommendation of a standard inclusion of serum biomarkers in the algorithm for selection of patients with HCC for LT.

Response to neoadjuvant treatment and evolution while on the waiting list

Some authors such as Otto et al[5] have used the response to neoadjuvant treatment as a selection criterion. In this study, 96 patients with HCC, 62 of them with staging that exceeded the Milan criteria, were treated with repeated CHE. Of these, only those patients that achieved downstaging were put on the waiting list. In the end, 50 patients received transplants (34 with expanded criteria). The patients whose tumours did not progress during their time on the waiting list had a 94.5% survival rate 5 years after the LT, while those cases that showed progression following the initial response presented a 35% illness-free survival rate at 5 years.

In the multivariate study, the risk of recurrence turned out to be dependent on tumour progression while on the waiting list and the number of nodules, whereas Milan criteria had no impact.

According to these results, large or multifocal HCCs could be treated by LT if they show a positive response to CHE and remain stable during sequential treatments.

Tumour histology

The third procedure used for identifying prognostic factors, and that we will develop more in depth, is based on preoperative histological studies using liver biopsies. A revision of the published literature on the subject leads to the conclusion that vascular invasion is the most relevant prognostic factor. When the vascular invasion is macroscopic, in many cases it can be detected by imaging techniques and is considered to be an absolute contraindication for LT. Microscopic vascular invasion, on the other hand, cannot be detected preoperatively.

The only conventional histological information that is detectable in the biopsy and potentially related to the aggressiveness of the tumour is the grade of differentiation. As a result, strong interest exists in confirming whether or not this variable allows precise predictions on the existence of a microscopic vascular invasion, whether on its own or in combination with other variables of grade of differentiation. If this were possible, could LT be contraindicated by an elevated risk of microscopic vascular invasion? Naturally, another valid question would be whether or not the same grade of differentiation could be useful in patient selection.

Is it possible to predict the presence of microscopic vascular invasion?

In 2001, Jonas et al[6] published a retrospective study performed on 120 patients with HCC transplantations with cirrhosis. Microscopic vascular invasion was observed in 48 of the 120 patients, and was associated with a significantly lower survival rate than in the rest of the patients, close to 50% at 5 years. The multivariate analysis indicated that histological grade and size were significant predictive factors for vascular invasion. According to the results of this study, microscopic vascular invasion could be considered as a contraindication for LT, and the combination of size and grade of differentiation could be useful for predicting its presence. The results obtained in a study by Esnaola et al,[7] published one year later, are similar and confirm that size and differentiation are the most relevant prognostic factors for vascular invasion; as such, they suggest performing a preoperative biopsy in order to detect the grade of differentiation. On an isolated basis, a size greater than 4 cm or a poorly differentiated tumour would implicate a 50% risk for the presence of microscopic vascular invasion. By analysing both size and grade of differentiation (tumours larger than 4 cm and poor differentiation), one can identify a group of patients with a 61% risk of vascular invasion.

Using these and many other previous experiences with similar results, Shirabe et al[8] put together a recently published scoring system. In a retrospective section of the study conducted using resection parts, they found that the histological grade, size, and des-gamma-carboxy prothrombin levels are the most relevant predictive factors for microscopic vascular invasion. With these 3 factors, they elaborated a score and put it to test in a retrospective study with another group of 32 patients with transplantation for HCC. A score of 3 or higher was associated with the presence of vascular invasion in 89% of cases, with only 21% in patients with scores lower than 3.

Would an elevated risk of microscopic vascular invasion contraindicate a liver transplantation?

In a recent (2007) study published by Lohe et al[9] on the results of 97 LTs, they discovered microscopic vascular invasion in 23 cases (24%). These patients had a 5-year survival rate significantly lower than the rest of the patients (7% vs 52%). However, results from other studies with this type of patient, although worse, justify maintaining the LT indication. In a study of 155 cases published the same year by Shah et al[10] and with a similar incidence of microscopic vascular invasion, the 5-year survival rate was greater than 60% even in the presence of this histological factor.

Probably, one of the key points for interpreting this difference is the results from a recently published study by the Metroticket Investigator Study Group.[11] This study clearly shows how the biological aggressiveness of the tumour, reflected in the presence of vascular invasion, influences survival. When this is not present, the macromorphological selection criteria can be broadened to include the up to seven rule, which is the sum of the largest size of the tumour in centimetres and the number of nodules. In contrast, when vascular invasion is present, the number and size of the nodules must be further restricted. The presence of vascular invasion is associated with inacceptable survival rates when the largest tumour surpasses 5 cm in diameter. Under this size, the results are reasonably good. As a result, it is plausible that in those studies where the mean tumour size is low, the influence of vascular invasion on survival is less clear.

What were the tumour sizes in the previously mentioned studies by Lohe and Shah? In the first, 54 tumours (56%) were larger than 3cm, and 30 tumours (31%) were larger than 5 cm. In the Shah study only 15 tumours (10%) were larger than 5 cm and the mean size was 2.6 cm. As a result, we can deduce that the tumours from the Lohe study were larger in size, implicating a worse prognosis, as previously mentioned.

These 3 factors (size, vascular invasion, and grade of differentiation) were used in some degree by Parfitt et al[12] in a study published in 2007, in which post-LT recurrence is predicted using histology results from the hepatectomy. In the multivariate analysis, the independent factors for prognosis of recurrence following LT were: microscopic vascular invasion, size, presence of microsatellites, and an aspect directly related to cellular differentiation, the presence of giant cells. With these data they created a scoring system with 3 levels of risk. Using this score, the results were confirmed that even in the presence of vascular invasion, a tumour size < 3 cm implies a low risk of recurrence (< 5%).

One conclusion is that the presence of vascular invasion does not represent a contraindication for LT, as long as the patient is within the Milan criteria. However, the contraindication would exist in the case that the patient surpasses the Milan criteria. A preoperative biopsy could contribute a high-precision evaluation of the risks of microscopic vascular invasion.

What information exists on the strength of the grade of differentiation as an independent prognostic factor?

Klintmalm,[13] in 1998, published an analysis of the data from the International Registry of Hepatic Tumours in Liver Transplantation that included 422 patients.

In the results, patients with differentiation grade 3 and even more so grade 4 tumours presented clearly lower survival rates than patients with grades 1 or 2. In the univariate analysis, the factors that influenced survival were tumour size > 5 cm, vascular invasion, the presence of ganglia + and histological grade. In the multivariate analysis, the authors demonstrated that only histological grade has a negative impact on survival. An especially striking result is that in the group of patients with well-differentiated tumours, size and vascular invasion do not seem to influence survival. The

authors conclude that LT can be contraindicated in patients with poorly differentiated tumours and a biopsy would be needed in order to make a decision in this case.

The study by Tamura et al,[14] published in 2001, includes only 53 cases, but all of these were evaluated in the same centre by the same pathologist. In the multivariate analysis for risk of recurrence, only grade of differentiation and tumour size were significant variables, while vascular invasion lost statistical significance. The 3-year survival rate in patients with well-differentiated tumours greater than 5 cm was 62.5%, while survival in patients with undifferentiated tumours was 0%. In contrast, no significant differences according to histological grade were found in patients with tumours smaller than 5 cm. Once again, these results indicate that tumour size modulates the effect of histological variables. Biopsies do not seem to be necessary in cases of tumours smaller than 5 cm, but a preoperative biopsy could be indicated for determining histological differentiation in cases of larger tumours.

In 2004, Cillo et al[15] published the results from the University of Padua on HCC patients for LT. In the preoperative study, a systematic FNP was performed to establish the grade of differentiation on a scale of 1 to 3. Among the LT exclusion criteria was a level 3 grade of differentiation. In the end, 33 patients received transplants. Thirty-eight percent of the cases did not comply with the Milan criteria in the surgical specimen. Vascular invasion was 4% in cases of grades 1 and 2 in this study. In the follow-up period (mean 44 months) only 2 cases experienced recurrence, both during the first year and having complied with the Milan criteria before the LT. The final conclusion is that the grade of differentiation is a marker for biological aggressiveness independent of vascular invasion.

Thus, numerous possible evidences exist regarding the possible usefulness of histological grade as a predictive factor for evolution. However, in spite of these and other results, no authors have investigated this topic in a prospective study. In part, this is related to the possible complications of the biopsy that, along with the risks of haemorrhage, include the possibility of tumour spread and lack of significance.

The possibility that the biopsy or a FNP of a tumour nodule might produce implantation of tumour cells along the trajectory of puncture has been widely studied in the medical literature. From a revision of articles published on this subject, we can conclude that this implantation occurs in 0-2% of cases, depending on the calibre of the needle and the technique.[16] When this procedure is accompanied by a percutaneous treatment such as radiofrequency, this rate is lowered (0.6%) and is practically null when the coaxial technique is employed.[17] Evidence also shows that a higher frequency of tumour recurrence exists following LT when a tumour biopsy is performed.[18]

Secondly, the significance of the biopsy with respect to a definitive anatomopathological report is unclear. In a publication by Pawlik in 2007,[19] a retrospective study was performed on 93 patients on whom a preoperative biopsy and resection were performed. Of the 93 cases, there was a complete concordance of the grade of differentiation (G1, G2, and G3) between the preoperative and postoperative reports in only 42 cases (45%) ($K = 0.18$; $P<.0001$). The study was repeated grouping G1 and G2: following this change, concordance increased to 76%, although the K-value continued to be significant. The conclusion was that the preoperative grade of differentiation had no significance compared to the habitual macromorphological parameters, and therefore, these must continue to be used as main selection criteria for patient selection.

One of the motives for explaining this apparently low predictive capacity of a preoperative biopsy is the heterogeneity of HCCs, which increases with their size.

Taking into account the limitations of histological parameters in recent years, the prognostic value of various biological molecular markers has been researched.

What can new understanding of molecular biology of hepatocellular carcinomas bring?

The methodology for studies of the prognostic value of genetic expression in relation to HCC is similar to that used for histological data. These are retrospective studies that generally employ paraffin-embedded samples that attempt to establish the prognostic value of various HCC molecular markers. This is the case of an article published by Chua et al[20] in 2007 regarding the *NDRG1* gene. The protein created by this gene participates in regulation processes for cell growth and differentiation. The results demonstrate that the over-expression of this gene is associated with a lower survival prognosis (< 20% at 5 years), and the multivariate analysis shows that this is a significant independent prognostic factor. Unfortunately, this study does not provide information on the treatment applied, or the cause of death, and includes 28 cases with tumours greater than 8 cm in diameter. As a result, as in many other cases, this lacks clinical applicability in the context of patient selection for LT.

Given the high level of heterogeneity in the processes of hepatocarcinogenesis, the establishment of a molecular classification system or a joint study of the expression of various genes seems to offer more possibilities of facilitating a reliable prognosis. Furthermore, it would be preferable that these studies be performed with cohorts of carefully selected patients susceptible to radical treatments, and a clear establishment of tumour-caused death as an end-point.

In the article by Iizuka et al,[21] a score is developed based on the expression of a group of 12 genes from a sample of 33 patients in which a curative HCC resection had been performed. Subsequently, they tested the ability of the expression of these genes to predict recurrence within one year in another group of 27 patients. The prediction was correct in 25 of 27 cases. The positive predictive value (PPV) was 88% and negative predictive value (NPV) was 95%, while the presence of venous invasion only had a PPV of 64%. However, we know that LT allows a better control of the tumour than resections for the same tumour stage, and thus necessitates confirmed prognostic variables for transplanted patients. In a study by our group[22] using a commonly used immunohistochemical technique for detecting the level of

expression of the *pRb* gene, we found that patients who received transplantations for HCC had high *pRb* positivity associated with a 100% recurrence within 6 years.

Recently, a study published by the University of Pittsburgh and the Mount Sinai Hospital proposed an assessment of the fraction of allelic imbalance (FAI) as a prognostic parameter in patients with HCC and a possible selection factor for LT. The FAI is an index defined as the ratio between the number of mutated markers from amongst the possible informative markers. The FAI represents a crude assessment of accumulated mutational damage. In this study by Dvorchik et al, 9 possible markers are employed from a panel of 18 suppressor genes.

In the multivariate analysis of illness-free survival, the FAI was the most significant prognostic factor, such that an FAI > 40% implied an increase in the risk of recurrence 19.5 times compared to an FAI < 20%, while the presence of a macrovascular invasion only increased the risk by 5.9 times compared to its absence. Furthermore, in patients with an FAI < 20%, vascular invasion lost its prognostic value.

Conclusions

The role that biopsies might play in the selection of patients with HCC for LT remains undefined. At the moment, a standard procedure preoperative biopsy for determination of the grade of differentiation or evaluation of the risk of vascular invasion in patients with HCC that comply with the Milan criteria cannot be recommended. Some evidence indicates that patients with an expanded criteria but well differentiated tumours could benefit from LT. A biopsy would theoretically identify these patients, although its reliability is unclear. It is probable that in the near future, studies of HCC biological markers will offer more precise markers, making a preoperative biopsy unnecessary. We only have to await the results from these studies, probably already commenced, from the centres with the capacity to perform the necessarily complex translational research.

Conflict of interest

The authors affirm that they have no conflicts of interest.

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