ORIGINAL ARTICLE

Number of hepatocellular carcinoma nodules in patients listed for liver transplantation within alpha-fetoprotein score: a new prognostic risk factor

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SUMMARY

In France, the listing for liver transplantation (LT) for hepatocellular carcinoma (HCC) requires an AFP score ≤2. This study evaluates whether the number of nodules assessed immediately before LT has a prognostic value among patients already listed within AFP score. Among 143 recipients transplanted with an AFP score ≤2 between 2013 and 2017 in our center, the number of nodules was considered at listing on the waiting list and at last imaging before LT. We compared the overall survival (OS) and disease-free survival (DFS) post-LT of patients with ≤3 and >3 nodules (current classification), and aimed to propose a new criteria to exclude patients on list at high risk of recurrence. The 3-year OS of patients with ≤3 HCC vs. >3 HCC at listing was of 90.3% vs. 67.3%, respectively ($P = 0.04$). At last imaging, eight listed patients presented ≥5 HCC nodules and had a significantly lower OS than <5 nodules patients (5-year OS: 24.4% vs. 78.1%; $P = 0.01$). Although the current AFP score offers satisfactory outcomes, we highlight the poorer outcomes when ≥5 nodules persist or appear after listing. A modification of the AFP score is mandatory to consider exclusion of high-risk patients already listed for LT program.

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Introduction

Worldwide, liver cancer is the sixth most common cancer, and the fourth leading cause of cancer mortality. Hepatocellular carcinoma (HCC) is the most common primary liver cancer [1].

Liver transplantation (LT) is the best cure for HCC but, because of the organ shortage, this treatment remains restricted to patients with an expected minimal 5-year post-LT survival >50% [2]. Selection of HCC patients for LT has been initially established according to the Milan criteria (MC) (1 tumor <5 cm or 3 tumors <3 cm) for more than 20 years to decrease the risk of post-LT tumor recurrence. Patients within MC experienced an OS and DFS at 4 years of 85% and 92%, respectively, whereas patients outside criteria had an OS

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and a DFS at 4 years of 50% and 59%, respectively ($P = 0.01$ for OS; $P = 0.002$ for DFS) [3].

Recently, other studies have suggested that an expansion of tumor burden beyond the MC could achieve post-LT survival rate comparable to that within MC [4–6]. This concept of expanded criteria has been compared to the Metro Ticket system, with the paradigm of "the further the distance, the greater the price." [7] Among the proposed expanded criteria, the University of California, San Francisco (UCSF) criteria, and the Up-to-seven criteria are best known rules, while not used in France [5,8].

Although most countries adopted the Model for End-stage Liver Disease (MELD) score for prioritization in liver transplantation, the French allocation system has been created with MELD as a reference for prioritization, but it also takes into account the geographic distance between the donor and the recipient [9]. In addition, the French AFP model introduced by Duvoux in 2012 is composed of three subscores including the largest tumor diameter (≤3 cm = 0 point; 3–6 cm = 1 point, >6 cm = 4 points), tumor number (1–3 = 0 point; ≥4 = 2 points), and alpha-fetoprotein concentration in ng/mL (≤100 = 0 point; 100–1000 = 2 points; >1000 = 3 points), the cutoff for transplant eligibility being of ≤2 points [10]. So patients with HCC and AFP score 2 or less receive extra points to counteract their lower MELD score.

In a validation cohort, a score greater than two points predicted a marked increase in 5-year recurrence risk (50.6 ± 10.2% vs. 8.8 ± 1.7%; $P < 0.001$) and decreased survival (47.5 ± 8.1% vs. 67.8 ± 3.4%; $P = 0.002$) as compared to patients with ≤2 points.

In France, it is allowed to list a patient for LT if the AFP score ≤2, meaning AFP ≤100 (0 point), unlimited number of HCC nodules (two points), as long as each one is ≤3 cm (0 point). Independently, it is well known that the number of tumor nodules is, by itself, a negative predictor but it has not been clearly reported whether this number of nodules also has a prognostic value among patients within the AFP score. In fact, there is no upper cutoff for the number of HCC nodules within the AFP score. Our objective was to evaluate whether the number of nodules had prognostic value among patients transplanted strictly within Duvoux's criteria (AFP score ≤2). For that purpose, we assessed the impact of nodules number at listing and during the waiting period.

Patients and methods

All consecutive patients listed and transplanted for HCC in our tertiary center between 2013 and 2017 were retrospectively reviewed. We also analyzed the patients who dropped out while waiting for LT. The inclusion criteria were as follows: age >18 years old, HCC histologically proven on the native liver, AFP score ≤2 at listing, and LT. The exclusion criteria were coexistence of cholangiocarcinoma on explants, fibrolamellar carcinoma, or incidental HCC. The following variables were collected: demographic data, underlying liver disease and pathological characteristics of the tumor (number and size of viable HCC nodules, micro- or macrovascular invasion, and tumor differentiation), postoperative and long-term outcomes (recurrence and survival).

At listing, the number and size of the HCC viable nodules on radiological imaging, and the AFP level were taken into account. The same analysis was performed on the last imaging (CT, MRI) before LT.

After bridging treatment (treatment on the waiting list), tumoral response assessment was evaluated by RECIST (Response Evaluation Criteria in Solid Tumors) 1.1 criteria, based on tumor shrinkage [11].

The activity of hepatic inflammation and severity of liver fibrosis were graded according to METAVIR inflammatory activity and fibrosis stage. The grade of fibrosis as F0–F2 was defined as no or mild fibrosis, whereas F3 and F4 indicated severe fibrosis and cirrhosis, respectively.

All data were evaluated from the prospective French national database from the Agence de la Biomédecine (ABM), the review of every medical record and the multidisciplinary team meeting reports.

The patients were divided into several groups according to the number of nodules at the listing on the WL using the current AFP score classification (≤3 nodules vs. >3 nodules). Another cutoff was also sought and applied, based on the last imaging workup. Patients were followed to assess disease-free survival (DFS), and overall survival (OS) after LT.

The OS was defined as the period from the LT to death from any cause, or LT to end of follow-up (if patient still alive at the last follow-up). The DFS was defined as the delay between transplantation and the HCC recurrence or death (in the absence of recurrence). In the absence of HCC recurrence or death, the DFS was considered as the delay between transplantation and the end/loss of follow-up.

The recurrence was diagnosed on biological tests (AFP increase) and/or typical image (CT scan or MRI) and/or histologically proven HCC lesion.

During the study period, standard immunosuppressive therapy consisted of tacrolimus + steroids for 3–6 months post-LT ± mycophenolate mofetil according to local policy.

Patients were followed until death, or loss of follow-up (end of monitoring in October 2020). Tumor recurrence was closely monitored: serum AFP levels, liver ultrasound, and/or thoraco-abdominal CT were performed every three months during the first two postoperative years, then twice a year and/or when clinically indicated. Additional imaging (magnetic resonance imaging MRI, PET-CT) was performed when clinically/biologically required.

Statistical analysis

All quantitative data are expressed as mean (±SD) or median [range]; qualitative data are expressed as frequency (percentage). The Mann–Whitney *U* test for continuous variables and Pearson's chi-square test were used when applicable, or Fisher's exact test for categorical variables. Survival rates were calculated using the Kaplan – Meier method, and groups were compared with the log-rank test.

Statistical analysis was performed using R 3.6.2 (Core Team, 2019), the next R packages were also used for statistical analysis dplyr 0.8.5 (Hadley Wickham, Romain François), survminer 0.4.8 (Kassambara), survival (Terry M.)

Results

Dropped-out patients

Among the 196 patients listed for HCC from 2013 to 2017, 36 (18.4%) were not transplanted due to drop-out. The causes of drop-out were:

1. patient's death during waiting time in 32 cases (16.3% of listed patients). The mortality on the WL was either due to liver causes (liver failure and/or tumor progression) in 22 cases or due to extra-hepatic causes in 10 cases,
2. progression of tumoral disease (occurrence of tumoral portal thrombosis, extra-hepatic metastases, increase of the size of the tumor or increase in AFP levels outside AFP score) in three patients (1.5% of listed patients),
3. primary lung cancer occurrence for one patient.

Seventeen patients, still listed and waiting for LT at the end of the study period, were not considered in the analysis.

Epidemiological and perioperative features of transplanted patients

The 143 transplanted patients had a mean age of 63.3 years (SD ± 7.4). The preoperative clinical data and the main characteristics of the cohort are illustrated in Table 1. The two main etiologies of the underlying liver disease were alcohol consumption and HCV infection in 41.3% and 27.9% cases, respectively. At the listing on the WL, 16 (11%) patients presented >3 HCC; at the last imaging workup before the LT, 17 (12%) patients presented >3 HCC nodules (although being still within AFP score).

Pre-LT imaging after enrollment included repeat CT and/or MRI, only 7% of patients did not repeat any imaging after listing (because of short delay before LT). The median waiting time between listing and LT was of

Table 1. Epidemiological features of transplanted patients.

	Whole cohort (n = 143)	P value
Age at LT*	63.3 ± 7.4	
Sex (M/W)†	118 (82)/25 (17)	
Underlying hepatopathy†		
Alcool	41.3	
Hepatitis C	27.9	
Hepatitis B	11.9	
NASH	10.5	
Other‡	4.9	
HCC on normal liver	3.5	
Patients with >3 nodules†		
Listing on the WL	16 (11)	
Last imaging before LT	17 (12)	
Pathology	41 (29)	
Neoadjuvant therapy†	n = 128/143 (89.5)	
Recurrence†	n = 25	
Recurrence if >3 nodules at last imaging before LT	5/17 (29.4)	P = 0.13
Recurrence if ≤3 nodules at last imaging before LT	16/126 (15.3)	
Recurrence if ≥5 nodules at last imaging before LT	3/8 (37.5)	P = 0.09
Recurrence if ≤4 nodules at last imaging before LT	18/135 (13.3)	

HCC, hepatocellular carcinoma; LT, liver transplantation; M/W, man/woman; NASH, Nonalcoholic Steatohepatitis; SD, standard deviation; WL, waiting list.

*Values are given as mean ± SD.

†Values are given as n (%).

‡Budd Chiari disease; Wilson disease, Autoimmune hepatitis, Hemochromatosis.

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7.3 months [0–77], while the median time between the last imaging to LT was of 2 months [0–44].

One hundred and twenty-eight (89.5%) patients had at least one preoperative treatment (transarterial chemoembolization $n = 83$, radiofrequency $n = 16$, surgical resection $n = 22$, other treatment $n = 22$), without any significant difference among the subgroups (≤3 vs. >3 nodules on native liver histopathology).

Pathological features of liver explant

A fibrosis ≥F3 was represented in 121 cases (85%). The largest active nodule had a mean diameter of 17.4 ± 12.9 mm. Macroscopic vascular invasion was present in three patients (2%), while a microscopic vascular invasion was present for 42 patients (29%).

The differentiation grade according to Edmondson–Steiner staging (E) was: 32 (22.4%) patients had grade E1, 53 (37%) patients had grade E2, 26 (18.1%) patients had E3, 1 (0.7%) patient had E4 (Table S1).

Analysis of overall and disease-free survivals of transplanted patients

The median follow-up of the whole cohort was of 44 months [range: 4.1–86.5 months]. The entire cohort had a 3- and 5-year OS of 87% and 75%. The 3- and 5-year DFS were of 77% and 71%.

Cutoff three nodules at listing (current classification)

Considering the number of nodules at the listing for LT, the OS at 3 and 5 years were of 90.3% and 78.3% for patients with ≤3 HCC nodules. For patients with >3 HCC nodules, but still in the AFP score at listing, the 3-year OS was of 67.3% ($P = 0.04$), without any surviving recipients at 5 years post-LT (Fig. 1a). The 3- and 5-year DFS were of 79.6 and 72.3% for patients with ≤3 HCC nodules vs. 63.7% (3 years), without any recipients free from recurrence at 5 years post-LT for patients with >3 HCC nodules ($P = 0.29$) (Fig. 1b). Note that the median follow-up of patients with >3 nodules at listing was of 23 months, thus preventing to draw formal conclusion about long-term outcomes.

New cutoff of five nodules during waiting period

Considering the number of nodules at the last imaging workup before LT, the OS at 3 and 5 years were of 89% and 77.9% for patients with ≤3 HCC nodules vs. 83.6% and 50% for patients with >3 HCC nodules, still within AFP score ($P = 0.30$; Fig. 2a). The DFS at 3 and 5 years were of 80.9% and 73.9% for patients with ≤3 HCC nodules vs. 49% and 49% for patients with >3 HCC nodules, respectively ($P = 0.09$; Fig. 2b).

We looked for the best cutoff of HCC nodules, after listing, to discriminate patients with lower 5-year OS (using the minimum P value approach) and we observed that the most efficient cutoff of nodules number at last imaging prior of LT was of 5.

Eight patients presented at last imaging workup ≥5 HCC nodules (AFP score still ≤2 at LT). They presented a median follow-up post-LT of 38.3 months and had a significantly lower OS than patients with <5 nodules (3 years OS: 89.6% if <5 HCC vs. 72.9% if ≥5 HCC; 5 years OS: 78.1% if <5 HCC vs. 24.4% ≥5 HCC; $P = 0.01$; Fig. 3). As well, their 3- and 5-year DFS were shorter than <5 nodules patients: 79.9% and 73.4% if <5 HCC vs. 38.9 % and without any recipients free from recurrence at 5 years post-LT for patients with ≥5 HCC ($P = 0.06$). Among these eight patients, only three presented ≥5 HCC nodules at the listing, meaning that five patients progressed on the waiting list in spite of preoperative therapy (intraarterial chemoembolization) but they still remained within the criteria for LT. Among these eight patients with ≥5 nodules at last imaging, only one patient presented a significant increase of serum AFP level since the listing.

At listing, the cutoff of five nodules (six patients) did not dichotomize the OS between groups ($P = 0.80$). Among these six patients, only three still presented ≥5 nodules at the last workup; the other ones having been downstaged by the neoadjuvant treatment.

Discussion

Statement of principal findings

This study aimed 1/ to study outcomes of patients transplanted within current criteria, but bearing >3 nodules, and 2/ to establish a new threshold of the number of HCC nodules during the waiting time before LT to identify patients with poor oncological results (5-year OS <50%, as this rule is generally admitted as the limit to preclude patients from transplanting).

In this cohort, patients with more than three nodules at listing presented shorter OS and DFS, in spite of AFP score ≤2. However, it was not possible to definitively conclude about long-term survival (no survivor at 5 years in this series) because of the short follow-up and small sample size. The current AFP score must remain strictly applied.

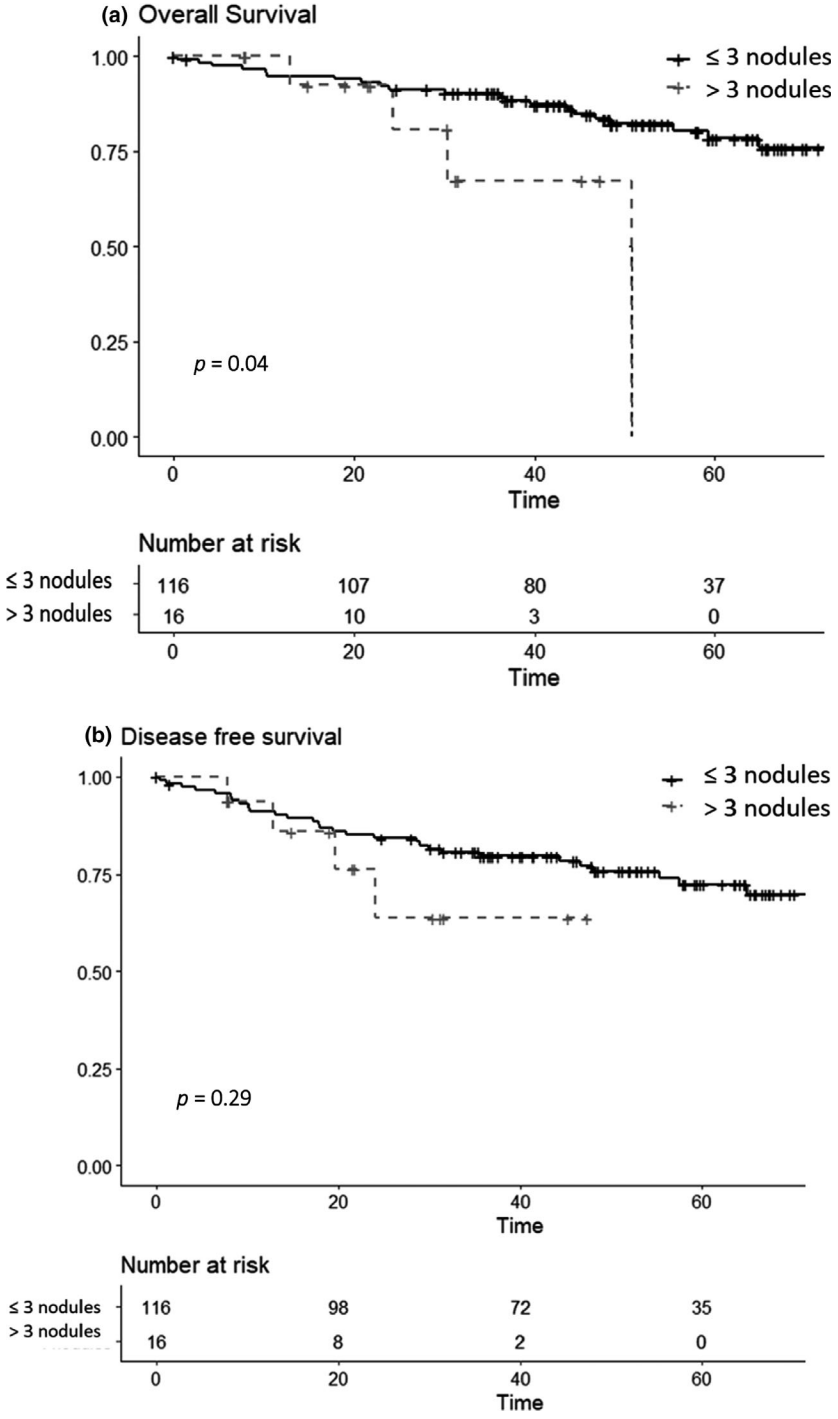

Figure 1 (a) Overall survival; (b) disease-free survival for patients with ≤ 3HCC nodules (bold line) vs. >3 HCC nodules (dashed line) at the listing on the WL for LT.

For patients stringently selected according to the AFP score, the presence of more than 5 HCC nodules during the waiting time on the list (whatever the number at listing) appears as a major predictor of tumor recurrence and poor survival after LT. Clearly, this cutoff (≥5 nodules) could not be applied at listing, because patients who responded to downstaging (after listing) had a good post-LT prognosis.

Our study emphasizes the importance of a dynamic (two steps validation) assessment according to tumor progression or tumor burden downstaging during the waiting time for transplantation, rather than a static

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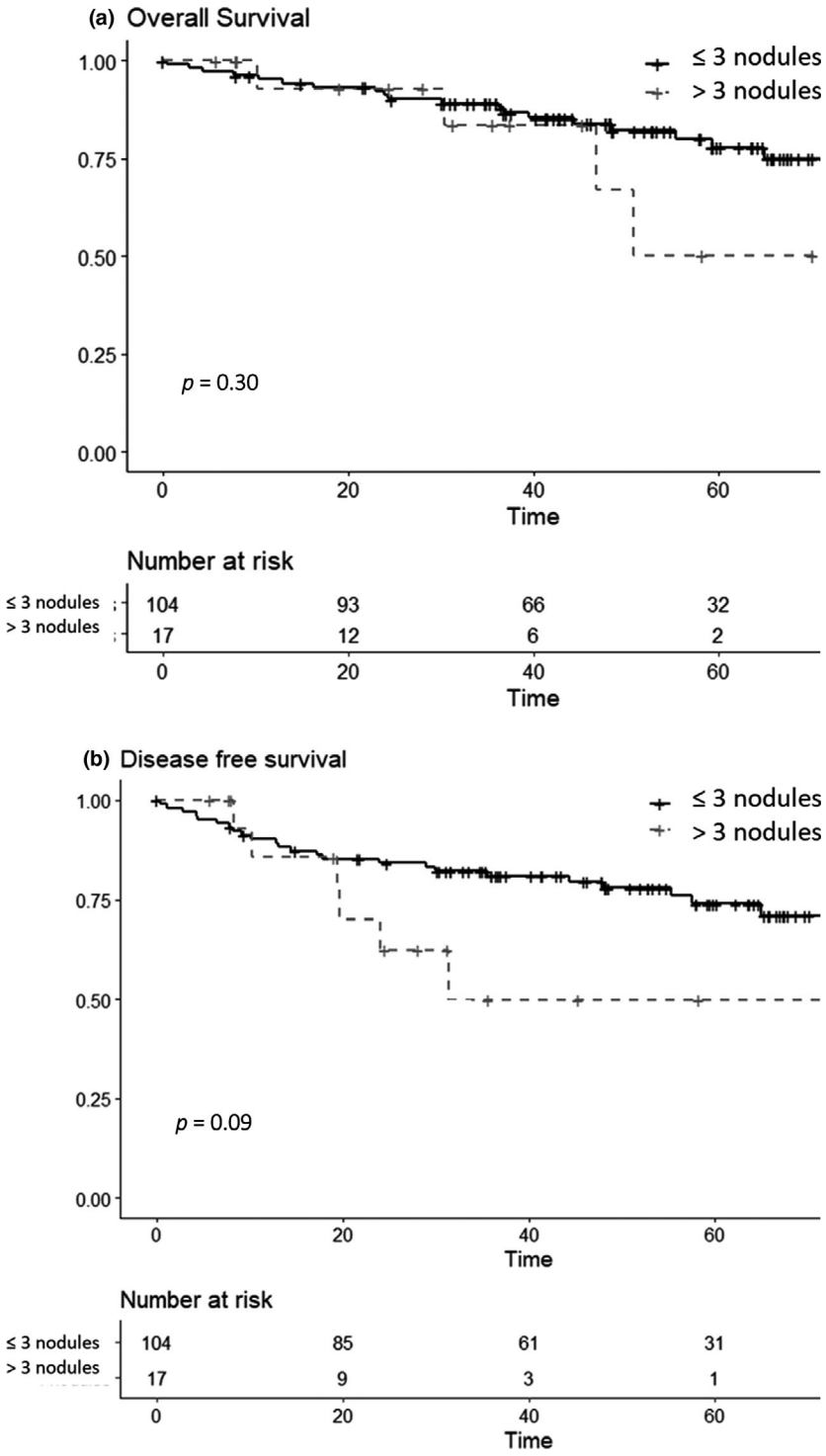

Figure 2 (a) Overall survival; (b) disease-free survival for patients with ≤3 HCC nodules (bold line) vs. >3 HCC nodules (dashed line) at last imaging before LT.

evaluation at the listing on the WL only. In fact, there was a huge discrepancy between criteria applied at listing or later. Among the 17 patients with >3 nodules at last workup (5-year OS of 50%), only seven presented >3 nodules at listing, meaning that 10 patients progressed on the list. The difference observed in OS rates between the 16 listed patients with >3 nodules and the 17 patients with >3 nodules at last imaging may be explained by a longer follow-up in the farmer group (23 vs. 31 months, respectively, $P = 0.15$).

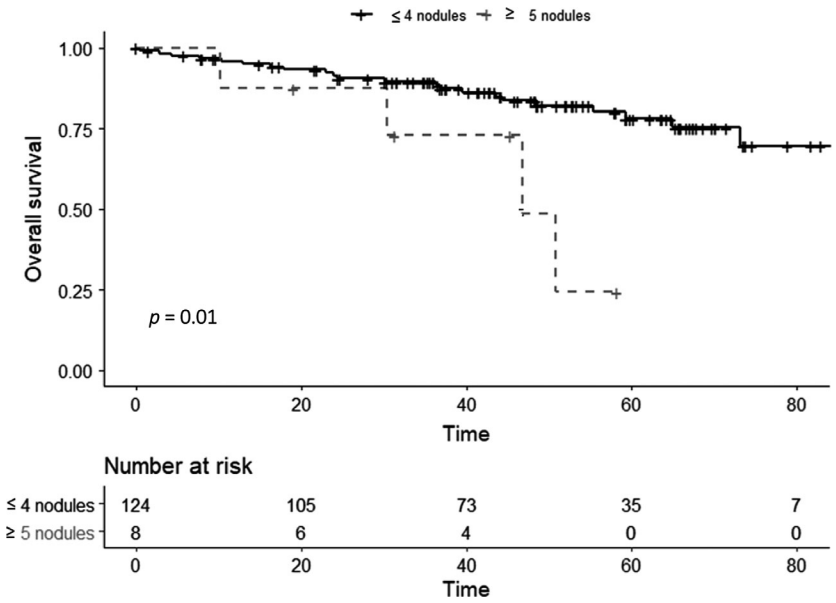

Figure 3 Overall survival for patients with ≤4 HCC nodules (bold line) vs. ≥5 HCC nodules (dashed line) at last imaging before LT.

It appears that if there is good control of the disease due to neoadjuvant therapy, there will be an acceptable prognosis. On the other hand, if the neoadjuvant therapy does not provide the desired oncologic control, there will be a worse prognosis. This "dynamic assessment" was more specific than any static evaluation of candidates at listing on the WL, and it could potentially be used to reappraise the indication of LT or the pre-transplant strategy. It could allow avoiding futile LT of high-risk patients listed according to current guidelines.

Patients listed within the AFP who have a number of nodules that increases during the waiting time to five or more HCC nodules before the LT have a poor prognosis. On the other hand, patients who are listed with five or more nodules within the AFP criteria, and who respond well to bridging therapy during the waiting time (shift to <5 nodules) can be considered as patients with a good prognosis according to our results. This highlights the usefulness of our score applied in a dynamic sense.

At last imaging, eight listed patients presented ≥5 HCC nodules and had a significantly lower OS than <5 nodules patients (5-year OS: 24.4% vs. 78.1%; $P = 0.01$). In our patient series, these patients would represent only 6% (8/143) of transplant patients. Although it is a small number, in our opinion it is important to highlight this negative trend in post-LT prognosis. In fact, the AFP score is already an effective predictive score, generally accepted. Our proposal marginally impacts the number of transplanted patients (6%), but considering that the HCC represents about 40% of indications for LT [12] this would result in sparing about 30 liver grafts per year in France, which could be relocated to other indications with better results.

To assess this potential benefit in terms of public health, it would be useful to carry out studies on a larger scale and confirm our preliminary results.

Interpretation with reference to other studies

Currently, the number of patients transplanted for HCC is increasing, with LT for HCC almost representing 50% of all LT performed in most centers [13]. Due to the current organ shortage, we need to refine our patient's eligibility criteria or LT that are very heterogeneous throughout the world; they usually combine variables such as the number of nodules, the size of the largest nodule, and the AFP level. However, none of these introduced a dynamic parameter of markers of tumor aggressiveness, as Vibert *et al.* [14] did: the authors emphasized a dynamic parameter (AFP increase >15 μg/l/month) as more accurate to predict recurrence than any increased static value of AFP in patients with HCC awaiting LT. In our series, the AFP variation (after listing) did not appear as an efficient predictor. This may be explained by their earlier study time, the features of their listed patients were much different from our cohort. As an example, the median number and size of HCC lesions in their paper were 2.3 and >3 cm, respectively, whereas we reported two lesions and 2.5 cm maximum diameter. The median waiting time on WL

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in their cohort was <6 months, shorter than ours. As well, the median pre-LT AFP value in our cohort was <10, much lower than in Vibert's report. Our study may be seen as an update of the initial concept of Vibert *et al.*, with recent cohort and modern HCC management.

Since January 2013 in France, the AFP score proposed by Duvoux *et al.* [10] is used for graft allocation. Even if many series validated this score [15–17], and while it is clearly established that the number of nodules is an independent predictor for post-LT prognosis, this score allows patients with multiple small HCC to be transplanted. We aimed to assess if this rule could also be applied after listing, and if integrating a tumor burden evolution before LT could help to select good candidates. Moreover, it is known that an imaging progression during the waiting period was a strong predictor of HCC recurrence, even in patients who met the Milan criteria [18]. However, a number threshold beyond 4 was never used to preclude LT in patients with HCC within AFP score ≤2.

A meta-analysis of fifteen studies (4575 patients) evaluating the impact of the number of tumor nodules on OS and DFS demonstrated that HCC nodules, evaluated as a continuous variable, did not have a clear impact on OS or recurrence, and when the HCC nodules were considered as a cutoff, the impact of three or more tumors on DFS and recurrence after liver transplantation was not significant. This study concluded that using a strict HCC nodules number as a cutoff should be avoided and a system taking into account larger lesions (e.g., a diameter ≥1.5 cm) might be better [19]. Our dynamic parameter (listing according to AFP score, confirmation during the waiting time with another threshold) could potentially be used to reevaluate the indication of LT or the pretransplant strategy, due to its significant prognostic role.

We showed the discrepancy between the number of nodules diagnosed at the listing on the WL, those detected during the last imaging before LT, and those founded on the pathology of the explanted liver, this indicating a global underestimation of the disease before the LT. This misevaluation between the number of HCC nodules as assessed on pre-LT imaging and explant pathology has been already reported [20] and according to this study the best predictors of HCC recurrence was the combination of pre-LT AFP >100 IU/l and diameter of the biggest nodule >3 cm at the radiological imaging.

In our cohort, considering the number of HCC nodules: eight patients with more than five nodules at last imaging either progressed compared to enrollment (<5 nodules at listing, $n = 5$), or insufficiently downstaged (already more than five nodules at listing, $n = 3$). These patients had poorer prognosis in terms of OS and DFS. Probably these patients would have required a better oncological control.

Same results were also shown by another study where radiological progression while on the waiting list was a strong predictor of high HCC recurrence: the 3- and 5-year OS rates were 65.5% and 48.9% for progressive group versus 84.8% and 74.6% for complete/partial or stable group ($P = 0.01$). The 3-year and 5-year DFS rates were 74% and 74% for progressive group and 95.7% and 93% for complete/partial or stable group ($P = 0.007$) [18].

Several studies [21,22] have focused on the difference between radiology and histopathology of the liver explant and it is known that pretransplant radiological staging fails to predict the number of HCC nodules on liver explant in about 25–35% of patients [23,24]. The underestimation at imaging workup prior the LT emphasizes the fact that it is necessary to refine the pretransplant staging. Current imaging requirements for radiological staging before LT are unacceptably inaccurate and the policy should require more accurate modalities or combinations of techniques [25]. So, our study has also the interest of introducing a new cutoff during waiting time (≥5 nodules) which could be used to reassess the risk of HCC recurrence post-LT.

Strengths and weaknesses of the study

The present study has several limitations: first, this is a retrospective study. However, it was carried out in a tertiary high-volume center and it reflects modern HCC management. Because this is a recent series, the follow-up is reduced and prevents from drawing long-term analysis.

However, the advantage of our study is the use of current and homogeneous enrollment criteria (AFP score ≤2), and the use of registers obtained from prospectively maintained database with comprehensive data from the French biomedicine agency (ABM).

Due to the sample size and the current criteria applied (AFP score), this study was not designed to challenge the Duvoux's classification, but to refine it.

We did not consider patients who dropped out from WL, mainly because we aimed to predict post-LT recurrence (not necessarily the same risk factors as for the drop-out).

It has been well reported the correlation between pathological findings (number of nodules, grade of

differentiation, or vascular invasion) and oncological outcomes. Obviously, explanted-based prediction is a critical step in the management of patients after LT for HCC. A reassessment of the risk of recurrence after transplantation based on the explant pathology is important to refine prognosis, to evaluate adjuvant strategies, and to adapt screening policies, but it is not possible to propose a score based on liver explant pathology because, by definition, this score could not be applied before total hepatectomy. That is why we aimed to focus on preoperative data, especially noninvasive ones.

Conclusion

The current AFP score is still valid, but in our study, we highlighted the high risk of post-LT recurrence and death of patients who, while remaining in the AFP score criteria, had more than 5 HCC nodules during the waiting period. Our new cutoff, applied during the waiting period, could prevent from transplanting high-risk patients, otherwise inside current criteria.

This may reflect a progressing disease (or insufficient decrease of tumoral load) in spite of neoadjuvant treatment, meaning an aggressive tumor leading to a high risk of recurrence. Progression of HCC nodules during the waiting time for LT could be a marker of poor prognosis, useful to select patients for LT. A recalibration of the AFP score should be taken into account, considering the exclusion from the LT program for the cases with ≥5 HCC nodules during the waiting period for LT. This issue should be further tested on a large multicentric prospective study.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Pathological features.

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