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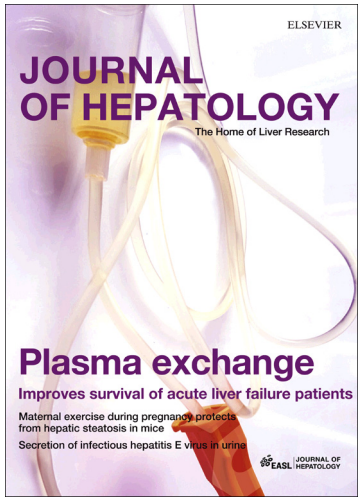

Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC

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Validation of the AFP model as a predictor of HCC recurrence in patients with viral hepatitis-related cirrhosis who had received a liver transplant for HCC.

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Key words : hepato-cellular carcinoma, liver transplantation, alfa foeto-protein, AFP, AFP model, AFP score, predictive model, validation.

Abbreviations:

ABM: Agence de la Biomédecine ; AFP: alfa foeto-protein ; HCC: hepato-cellular carcinoma ; LT: liver transplantation.

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Authors' contributions:
AN, CD and FRT designed the study; AN, AB, PM, MG, GM, LM, AV, GV, CDA, MC and GM carried out data collection; RL, SK and EA analyzed the results and conducted the statistical analysis; AN and CD drafted the publication. All authors significantly contributed to the manuscript.

Abstract :

Background and aims: The AFP model was shown superior to Milan criteria for prediction of HCC recurrence after liver transplantation in a French population. Our aim was to test the AFP model in a non French, post-hepatitic cirrhosis-based population of HCC candidates.
Methods: 574 patients transplanted for HCC in 4 Italian centres were studied. AFP score was assessed at last evaluation before LT. Probabilities of recurrence and survival were estimated by the log rank test or competing risk analysis and compared according to the AFP model.
Results: 24.7% pts were beyond Milan criteria. HCC complicated HCV and HBV cirrhosis in 58.7% and 24% of the cases. Five-year probabilities of recurrence differed according to AFP score ≤ 2 vs > 2 in the whole population (13.2±1.8% vs 49.8±8.7%, $p< 0.001$, HR=4.98), in patients within Milan criteria (12.8±2.0% vs 32.4±12.1%, $p=0.009$, HR=3.51), beyond Milan criteria (14.9±4.2% vs 58.9±11.5%, $p<0.001$, HR= 4.26), HCV patients (14.9±2.5% vs 67.6±14.7%, $p< 0.001$, HR=6.56) and HBV patients (11.6±3.4% vs 34.3±12.5%, $p=0.012$, HR = 3.49). By NRI analysis AFP score significantly improved prediction of non recurrence compared to Milan criteria.
Overall five-year survival rates according to AFP score ≤ 2 or > 2 were 71.7±2.2% vs 42.2±8.3% ($p<0.001$, HR =2.14).

Conclusions: The AFP model identifies HCC candidates at low risk of recurrence otherwise excluded by Milan criteria in a population with a predominance of post-hepatitic -related HCC. The AFP score can be proposed for selection of HCC candidates in programs with a high proportion of viral/HCV-related cirrhosis.

Electronic word count: 249 words

Key words: liver transplantation, hepato-cellular carcinoma, recurrence, AFP, AFP model, AFP score, predictive model, validation

Lay summary of the abstract

Selection criteria for liver transplantation of patients affected with hepato-cellular carcinoma (HCC) are based on Milan criteria which have been shown too restrictive, precluding access to liver transplantation of some patients who might be cured by this operation. Recently a French group of researchers developed a new selection model called the AFP model, or AFP score, allowing some patients with HCC not meeting Milan criteria to be transplanted with excellent results. In the present work, this AFP score was tested in a population of non French patients transplanted for HCC occurring mainly on post-hepatitic (HCV or HBV) cirrhosis. The results confirm that in this specific population, as in the original French population of patients, the AFP model better selects patients with hepato-cellular carcinoma eligible for transplantation, compared to Milan criteria. We conclude that the AFP score which has been officially adopted by the French organization for Organ Sharing for HCC patients can also be implemented in countries with an important burden of HCC occurring on post-hepatitic cirrhosis.

Introduction:

Liver transplantation (LT) is considered the best treatment of hepato-cellular carcinoma (HCC). However its efficacy is limited by the risk of tumor recurrence which results in rapid death and graft loss in patients who are not selected appropriately, making LT futile.

Because of this intrinsic limitation, considerable efforts have been attempted to select HCC candidates having the lowest risk of tumor recurrence. For this purpose, Milan criteria have been proposed 18 years ago (1), and have been adopted by a number of LT programs and centers around 2000, notably in the USA. Over the last decade yet, some groups have reported on expanded HCC criteria which were associated acceptable risk of recurrence, around 10-15% on average (2-9) and with 5-yr survival rates similar to those observed after LT for benign liver diseases. These findings indicate that some patients can be transplanted beyond Milan criteria with excellent results and point out that Milan criteria are probably too restrictive. However, no consensus has been achieved on such expanded criteria which were mostly derived from retrospective analysis of explant pathology with no prospective validation on external cohorts nor direct comparison to Milan criteria. Therefore, the 2010 International consensus conference on HCC and LT (10) stated that Milan criteria remained the benchmark for selection of HCC patients for LT, and the basis for comparison with any other suggested criteria. Yet, recommendation 10 (10) opened a door to expanded criteria, provided such criteria would not significantly affect LT for other benign indications.

Recently the French study group for LT reported on a new predictive model for HCC recurrence, namely the AFP model (11), which was based on tumor staging and AFP values at listing and follow-up time points. Adding AFP to tumor size and number increased the accuracy of prediction for recurrence because AFP is a surrogate marker of both tumor differentiation and vascular invasion (11-14), two features which cannot be assessed by conventional imaging-based tumor staging. Accordingly, high AFP levels have been reported to be associated with high recurrence rates (2-3, 11, 15-16). The AFP model was shown superior to Milan criteria to predict recurrence (11) in a training set of HCC patients and was subsequently validated in a cohort of 460 French patients followed prospectively under the control of the French Organization for organ sharing (ABM). On this ground, the AFP model was officially adopted in January 2013 I France by ABM for selection of HCC candidates. However, whether the AFP model may select appropriately non French HCC candidates, with different distribution of underlying liver diseases, remains unknown. As recently stated

by a European expert panel (17), incorporating a biomarker-based predictive model on a large scale deserves confirmation of results using the same technology in external cohorts reported by independent investigators.

The aim of this study was therefore to test the predictive value of the AFP model for recurrence and survival in an Italian population of HCC patients which differed from the French cohort by the predominance of HCC complicating post-hepatitic cirrhosis.

Patients and Methods

Patients:

The study population consisted of adult patients who had been listed and had undergone LT for HCC in the centers of Bergamo, Modena, Padova and Roma San Camillo between 2002 and 2010.

Inclusion criteria were (i) patients listed for HCC diagnosed either on preoperative imaging according to the EASL/AASLD criteria (17) or on pre-operative tumor biopsy, (ii) absence of tumor venous involvement on pre-operative ultrasound or CT scan examination of the liver; and, (iii) histo-pathological proof of HCC on the explanted liver.

Exclusion criteria were (i) incidental HCC; (ii) diagnosis of tumor vascular invasion on pre-operative imaging, at listing or during follow-up; (iii) diagnosis of HCC after listing; (iv) patients younger than 18 years of age.

A total of 684 patients were screened to participate this retrospective study. After exclusion of patients with missing data, the final study population consisted in 574 patients (Modena n=210, Padova n=168, Bergamo n=135 Roma n= 61), the characteristics of which are listed in table 1.

Methods

Data collection

1- Pre-transplant data at listing and post-transplant events

Demographics, cause of cirrhosis, MELD scores, imaging tumor features, type of pre LT bridging therapies, liver function tests and AFP values were retrospectively collected at listing and during the waiting phase by local investigators**. Imaging features of HCC had been collected from imaging reports. Diagnosis of HCC was based on EASL criteria (17). Response to treatment after loco-regional therapy was assessed according to mRECIST criteria, taking into account for size and number, the residual viable tumour**

tissue as assessed in the arterial phase of contrast-enhanced CT or MRI. Pathological features of HCCs, including vascular invasion, tumor differentiation, tumor size and number were collected after LT from pathological reports of the explants. Monitoring and modalities of diagnosis of HCC recurrence have been reported elsewhere (1, 18-20). Post LT follow-up data included death, cause of death, HCC recurrence and dates of last follow-up visit, death or recurrence.

Collection of data: **data were collected by independent local investigators blinded to the final data base and blinded to statistical analysis.** Data collection was supervised by AN.

2- Statistical analysis

AFP model: The AFP score was calculated for each patient enrolled in the study at listing and at last evaluation before LT, using the simplified version of the AFP model (11, table 1). However, due to a median waiting time of 8.6 months, data and AFP values closest from LT were eventually used to test the ability of the AFP model to predict both recurrence and death.

Probabilities of HCC recurrence and death were estimated and compared according to Milan criteria or the AFP score at a cut-off of 2 by the mean of the log rank test. Hazard ratio between low and high risk groups as defined by either the AFP model or Milan criteria were determined from univariate Cox models. Competing risks analysis (21) was used to compare the probabilities of HCC-related and unrelated deaths. Ability of Milan and AFP model to predict recurrence was also tested by the mean of net reclassification improvement analysis applied to patients with a minimal follow-up of 2 years (22). Analysis of histo-pathological features of HCC associated with recurrence post LT was performed by the mean of uni- and multivariate Cox models. Histo-pathological features of HCC were also compared according to AFP scores $>$ or $\leq$ 2 and Milan criteria.

The SPSS (V.18) and Stata (V11) softwares were used for statistical analysis.

Statistical analysis was performed by a team of statisticians (RL, SK, EA), independently of the investigators involved in data collection, after ruling out files with relevant missing data. In addition, the team of statisticians had not been involved in the design and validation of the original score and had no *a priori* on the expected end-points.

Results

Characteristics of the study population:

Baseline characteristics of the study population are presented in table 2. Median MELD score at listing was 12. The majority of HCC complicated post-hepatitic cirrhosis, i.e. HCV cirrhosis in 58.7% and HBV cirrhosis in 24% of the cases.

Of note, causes of liver diseases differed significantly in this cohort from those reported in the original French cohort (11) **with a significantly higher number of HCC occurring on post-hepatitic liver diseases and a lower number of HCC complicating alcoholic liver disease in the present cohort, compared to the French one (supplementary material, tables 1a & 1b).**

Assessment of HCC was performed by contrast-enhanced CT scan, MRI or contrast-enhanced US in 77.2%, 19.3% and 3.5% of the cases, respectively. Median time [IQ] from last imaging to LT was 2.2 [1.1-4.1] months. AFP value used for calculation of the AFP score was determined a median[IQ] of 5.4 [1.16-11.6] months after listing and 1.2 [0.4-2.8] months before LT. Twenty-five percent of HCCs were beyond Milan criteria at listing and AFP score was > 2 in almost 11% of the cases. Median waiting time was 8.6 months and 84.7% of the patients had received loco-regional bridging therapies during the wait phase, including thermo-ablation in 254 cases (associated with TACE in 111 cases, ethanol ablation in 65, and surgical resection in 22 cases), TACE in 160 cases, ethanol ablation in 23 cases and surgical resection in 67 cases (surgery only: 12, combined with loco-regional therapies: 55). Overall, post-operative mortality was 7.7%, and crude incidence of recurrence was 13.5%.

Probabilities of recurrence according to pre-LT AFP values.

5 year-probability of recurrence significantly differed according to pre LT AFP thresholds as defined by the AFP model (11), ranging from 13.0±1.8% to 34.9±6.8% and 75.0±15.3%, in patients with pre LT AFP values ≤ 100 ng/mL,]100 -1000 ng/mL] and > 1000 ng/mL, respectively (figure 1), p< 0.001.

Overall probabilities of recurrence and survival according to the AFP score cut off of 2 or Milan criteria

Five-year probability of recurrence, as assessed by Kaplan Meier (KP) estimates were 13.2±1.8% in 512 patients with AFP score ≤ 2 vs 49.8±8.7%, in 62 patients with AFP score > 2 (p <0.001, HR=4.98 [3.06-8.10]) (figure 2A). Accordingly, 5-year survival rates were 71.7±2.2% vs 42.2±8.3% (p<0.001, HR =2.14 [1.43-3.20]), among patients with AFP score ≤ 2 or > 2 (figure 2B)**,** indicating that in this cohort the AFP model discriminated appropriately between low and high risk patients for both recurrence and survival. These figures compared favorably with the risk of recurrence as assessed by Milan criteria (figure 2C and 2D). Risks of recurrence in patients within and beyond Milan criteria were 13.6±2.0% and 27.4±4.6%,

respectively (p<0.001), with corresponding 5-year survival rates of 73.5±2.3% and 54.3±5.0%, respectively (p=0.01)

. Of note, risks of recurrence as assessed by competing risk analysis, taking into account the competing risk of non HCC related death (Supplementary material, figures 1A and 1B) were estimated as 11.1±1.0% and 43.0±7.7% (p<0.001) in patients with AFP score ≤ and > 2, and 11.6±1.9% and 22.2±3.8% (p<0.001,) in patients within or beyond Milan criteria, indicating that KP estimates only slightly overestimated the risk of recurrence. Again these figures indicated that the AFP model better discriminated between high and low risk patients than Milan criteria. Finally, based on competing risk analysis, probabilities of death not related to HCC recurrence were similar, 20.6±1.9% and 20.1±5.8%, (p=0.76) in patients with AFP score ≤ 2 or >2 (figure 3) indicating that differences in survival rates according to the AFP model were actually due to HCC recurrence but not to other causes of deaths.

Probabilities of recurrence according the AFP score cut off of 2, in patients fulfilling or not Milan criteria (figures 4A and 4B).

Among 432 patients fulfilling Milan criteria, 5-year risk of recurrence was 12.8±2.0% in patients with AFP score < 2 and 32.4±12.1% in patients with AFP score > 2 (p=0.009, HR=3.51 [1.39-8.88]) (Figure 4A).

Among 142 patients beyond Milan criteria, the risk of recurrence was 14.9±4.2% among patients with an AFP score < 2 and 58.9±11.5% in patients with an AFP score > 2, (p<0.001, HR= 4.26 [2.10-8.67]) (Figure 4B). These results show that the AFP score was able to identify patients at low and high risk of recurrence both in patients fulfilling or not fulfilling Milan criteria.

Comparison of AFP model and Milan criteria according to net reclassification improvement.

Net reclassification improvement table for recurrence at 2 years is presented in supplementary table 2.

The AFP score significantly improved classification of patients without recurrence compared to Milan (NRI for non event = 0.137, z= 6.81, p< 0.001) confirming that AFP score performed better than MC to select patients at low risk of recurrence, even among patients exceeding Milan criteria. However, global NRI was not significantly different between the 2 scores (NRI=0.0303, z=0.434, ns) because NRI for event was similar for Milan criteria and AFP score.

Probabilities of recurrence and survival in HCV and HBV patients (figures 5 and 6).

In the subgroup of 337 patients transplanted for HCV-related HCC, the 5-year risk of recurrence was 14.9±2.5% in patients with AFP score ≤ 2 and 67.6±14.7% in patients with AFP score > 2 (p< 0.001, HR=6.56 [3.61-11.92]) (figure 5A). Corresponding 5-yr survival rates in HCV patients were 67.8±3.0% and 25.6±11.0% (p< 0.001) (figure 5B). **Similar results were found in** the subgroup of 138 patients transplanted for HBV-related HCC **in terms of recurrence**, (p=0.012, HR = 3.49 [1.23-9.93]) (figure 5 C) **although 5**-year survival rates according to AFP score did not achieved statistical significance (figure 5D).

Probability of recurrence and survival in 46 patients transplanted after down-staging from AFP score >2 to AFP score < 2.

The features of 46 patients with successful down-staging from AFP score > 2 to < 2 after loco-regional therapy are shown in supplementary tables 3a and 3b.

Median AFP scores before and after loco-regional therapy were 3 (3.00;3.00) and 0 (0.00;1.00), respectively; 30.4% of the patients remained out of Milan criteria but with AFP score<2 after down-staging. The majority of patients had been down-staged by the mean of percutaneous ablation techniques, in combination with TACE in nearly half of them.

The median time from down-staging procedure to transplantation was 81.00 (22.00;148.00) days that is nearly 3 months.

By competing risks analysis the 5-year risk of recurrence was 16.4±5.7% (supplementary fig2), with a corresponding overall 5-year survival rate of 71.8±7.0% (supplementary fig3)

The pathological features of tumours after down-staging are summarized in supplementary table 3b. Features of those tumours were quite similar to those of the whole group of patients with AFP <=2 in terms of number and size of nodules, and also for prevalence of micro-vascular invasion and poor differentiation on the explant (see below).

HCC Pathological features according to AFP score and comparison of AFP model and Milan criteria according to explant findings:

Explant-based tumor features according to AFP score are summarized in table 3. Multivariate analysis of histo-pathological predictors of recurrence show that micro-vascular invasion (OR 4.02 [2.51-6.44], p<0.001) and poor tumor differentiation (OR 1.98 [1.24-3.15], p=0.004) were significantly associated with the risk of recurrence. Risks of micro-vascular invasion and poor differentiation were higher in patients with AFP score > 2 than in patients with AFP score $\leq$ 2. In addition, tumor size was larger and tumor number was higher in patients with AFP score > 2 than in patients with AFP score $\leq$ 2

Comparisons of histo-pathological features of HCC according to Milan criteria and AFP scores are shown in **supplementary table 4**. In patients with AFP score > 2 (high risk of recurrence), prevalences of both micro- and macro-vascular invasion as well as poor differentiation were high and did not differ whether HCCs were in or out Milan criteria on the explant. In particular, prevalence of micro-vascular invasion and poor differentiation for HCC within Milan criteria but with AFP score > 2 were 46.7% and 60 %, respectively. This reflected a better association of the AFP score with high risk pathological predictors of poor prognosis, compared to Milan criteria. In patients with AFP score $\leq$ 2, prevalence of macro-vascular invasion and poor differentiation did not differ whether HCC were in or out Milan, here again indicating a better association of AFP score with these 2 pathological predictors of recurrence, compared to Milan criteria. Yet, in patients with AFP score $\leq$ 2, the prevalence of micro-vascular invasion was higher in patients beyond than within Milan criteria.

Discussion

Over the last decade, an increasing perception has emerged among the community of liver transplantation teams that Milan criteria, which have been adopted almost two decades ago as a selection tool for HCC candidates, have become too restrictive (10, 23) . However, although several new selection criteria have been proposed for expanding HCC indications (2-9), no consensus has been achieved so far for their use in clinical practice (23). In the present study we tested in an Italian population of HCC candidates the predictive value for recurrence of the AFP model (11), a recently proposed prognostic tool which has been designed in a French training cohort of HCC candidates and tested further in an external,

prospectively followed, validation set. The AFP model has been shown more accurate than Milan criteria for selection of HCC candidates in this French population and as so, has been adopted as an official selection tool by the French organisation for organ sharing (ABM) by 2013. Of note, the Italian cohort of HCC candidates differed from the French population because of a much higher proportion of HCC coming up on post-hepatitic cirrhosis with a 58% prevalence of HCV-related cirrhosis and 24% prevalence of HBV cirrhosis. Basically, the aim of this study was therefore to test the AFP model in an additional external cohort of HCC candidates which differed from the original one in order to ensure consistency. **Subject to the retrospective design of the study,** the results presented herein show that, as in the French cohort, the AFP model was able to discriminate correctly and more accurately than Milan criteria between patients at low and high risk of recurrence in the Italian, HCV/HBV-based population: the 5-year incidence of recurrence and probability of survival were significantly better among patients with AFP score $\leq$ 2 than in patients with AFP score > 2: 13.2±1.8% and 71.7±2.2% vs 49.8±8.7% and 42.2±8.3%, respectively ($p < 0.001$). In addition, competing analysis censoring HCC related deaths show that the 5-year incidence of HCC unrelated deaths were similar in patients with low and high AFP scores (19.0% vs 21.9%, ns). This finding demonstrated that better survival observed in patients with AFP score $\leq$ 2 was not due to a lower incidence of HCC unrelated deaths but actually to a lower incidence of recurrence.

The lower incidence of recurrence and higher survival rates in patients with AFP score $\leq$ 2 were observed whether patients met Milan criteria or not. In particular, among patients beyond Milan criteria, AFP score $\leq$ 2 identified a subgroup of patients with a low 5-year 14.9±4.2% risk of recurrence. On the opposite, among patients within Milan criteria, AFP score > 2 identified a subgroup of patients with a quite high 5-year risk of recurrence of 32.4±12.1%. This latter finding indicates that special attention should be payed to patients within Milan criteria and high AFP levels at listing before considering them fully eligible for transplantation. Indeed among such patients, those with AFP levels > 1000 ng/mL should be considered at high risk for recurrence, a finding already observed in the French cohort. A careful down-staging strategy to AFP score $\leq$ 2 in this subgroup of patients can reasonably be advised before considering LT. **Indeed, the results shown in the subgroup of patients who underwent a successful down-staging procedure before LT indicate that a reasonable risk of recurrence (i.e.16.4%) and excellent 5-year survival rate (i.e. 71.8%) may be achieved after down-staging to AFP score <2. Our** results also confirm that AFP brings up additional information about tumor behavior, compared to imaging staging, making possible the identification of aggressive tumors despite reasonable tumor size and number. Analysis of the relationship between AFP scores and histo-pathological features of HCC was

in agreement with this finding: HCCs with AFP scores > 2 had significantly more aggressive pathological features than HCC with scores < 2 .This was observed not only in the whole population but also in patients within Milan criteria: AFP score >2 was associated with 46.7% and 60 % prevalences of micro-vascular invasion and poor differentiation respectively in this subgroup of patients.

Interestingly, the AFP model performed in a population which differed notably from the French population in whom it has been developed and tested originally. A stated above, prevalence of HCC complicating post-hepatitic cirrhosis was > 80% in the Italian cohort vs 44% in the French validation set. However in the HCV population, accounting for almost 60% of the Italian cohort, 5-yr probabilities of recurrence were 14.9±2.5% vs 67.6±14.7% in patients with AFP score $\leq$ 2 or > 2 ($p< 0.001$) with corresponding highly different survival rates in this group (67.8±3.0% vs 25.6±11.0% in patients with AFP score $\leq$ or > 2 ($p<0.001$)), indicating that the AFP model prediction was independent of liver disease etiology and may therefore be applied in programs with a majority of HCV-related HCCs. The reason why the incidence of recurrence in the HCV population with AFP score > 2 was particularly high is unclear and further comparisons of pathological features of HCC in the HCV vs non HCV populations according to the AFP model are ongoing. A similar although less pronounced trend was observed in HBV-related HCCs. In this subgroup, 5-year HCC recurrence rate was significantly higher in patients with AFP score > 2 compared to AFP score < 2. However 5-year survival rate although lower in patients with AFP score > 2 did not achieved statistical significance. This might be due to the small number of patients in this subgroup with only 18 HBV patients with AFP score >2.

An important issue is also to determine whether adopting the AFP model may significantly impact the burden of HCC candidates and may further increase the competition with non HCC patients. The results presented herein show that in programs strictly adopting Milan criteria, expanding selection criteria to AFP model may result in a 14% increase in the number of patients eligible for LT (in this present series, 80/574 (14%) patients were beyond Milan criteria but had AFP score $\leq$2). However, denying LT to such candidates appears no longer ethical given the excellent, 72%, 5-year survival rate observed in the AFP score $\leq$ 2 patients. To balance the limited expansion of indications of LT for HCC resulting from adoption of the AFP model, additional allocation rules for HCC patients should be encouraged, based on baseline staging of HCC and responses to bridging therapies as recently implemented in the French program. On the other hand, in programs not strictly based on Milan criteria, the AFP model gives the opportunity of a better selection of high risk patients and therefore reduces the probability of futile transplantation for HCC. As so the

AFP model has recently been strongly discussed by the UK LT program for selection of HCC candidates (24). Recent data from Latin America also give additional background to support the use of the AFP model (25)

Although more accurate that Milan criteria for prediction of recurrence, the AFP score deserves further improvement. Some patients with AFP score > 2 do not recur and it is of utmost importance to identify them more specifically. Future research aiming at improving prediction of recurrence of HCC before LT is therefore mandatory. Extensive analysis of larger data sets, new predictive models integrating functional imaging (26-28) or/and molecular tools may overcome this issue in the future.

In conclusion, the AFP model which was designed in a French population also performs appropriately in an Italian cohort, characterized by a large predominance of HCV-related HCCs. As in the French population, the AFP model discriminates between patients with low and high risk of recurrence, both in patients within and beyond Milan criteria, indicating a better accuracy than Milan criteria for selection of HCC candidates. This study therefore shows that the performance of AFP model is reproducible and fulfills recommendations of the European expert panel (17) for incorporating AFP and the AFP model in the clinical management of HCC candidates. This important finding strongly supports the adoption of the AFP model as a selection tool for HCC patients in programs with a high proportion of HCC-related post-hepatitic/HCV-related cirrhosis.

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References

1. Mazzaferro VV, Regalia EE, Doci RR, Andreola SS, Pulvirenti AA, Bozzetti FF, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med. 1996;334:693–699.
2. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology. 2001;33:1394–1403.
3. Toso C, Trotter J, Wei A, Bigam DL, Shah S, Lancaster J, et al. Total tumor volume predicts risk of recurrence following liver transplantation in patients with hepatocellular carcinoma. Liver Transpl. 2008;14:1107–1115.
4. 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;7:2587-96.
5. Ito T, Takada Y, Ueda M, et al. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. Liver Transpl 2007;13:1637-44.
6. Herrero JI, Sangro B, Pardo F, et al. Liver transplantation in patients with hepatocellular carcinoma across Milan criteria. Liver Transpl 2008;14:272-8.
7. Onaca N, Davis GL, Goldstein RM, Jennings LW, Klintmalm GB. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma: a report from the International Registry of Hepatic Tumors in Liver Transplantation. Liver Transpl 2007;13:391-9.

8. Kneteman NM, Oberholzer J, Al Saghier M, et al. Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. Liver Transpl 2004;10:1301-11.
9. Mazzaferro V, Llovet JM, Miceli R, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol 2009;10:35-43.
10. Clavien PA, Lesurtel M, Bossuyt PM, et al. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. Lancet Oncol 2012;13:e11–e22.
11. **Duvoux C, Thoraval FR, Decaens T**, Pessione F, Badran H, Piardi T, et al. Liver Transplantation for Hepatocellular Carcinoma: A Model Including α-Fetoprotein Improves the Performance of Milan Criteria. Gastroenterology. 2012;143:986–994.e3.
12. Fujioka M, Nakashima Y, Nakashima O, Kojiro M. Immunohistologic study on the expressions of alpha-fetoprotein and protein induced by vitamin K absence or antagonist II in surgically resected small hepatocellular carcinoma. Hepatology 2001;34:1128-34.
13. Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Am J Transplant. 2009;9:2362-71
14. Liu C, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, Wang WT, Xu MQ, Yang JY. Value of α-fetoprotein in association with clinicopathological features of hepatocellular carcinoma. World J Gastroenterol 2013; 19: 1811-1819
15. Ravaioli M, Grazi GL, Piscaglia F, Trevisani F, Cescon M, Ercolani G, et al. Liver Transplantation for Hepatocellular Carcinoma: Results of Down-Staging in Patients Initially Outside the Milan Selection Criteria. American Journal of Transplantation. 2008;8:2547–2557.
16. Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. Liver Transpl. 2014;20:945–951

17. European Association for the Study of the Liver, European Organization for Research and Treatment of Cancer. EASL-EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma. Journal of Hepatology. 2012;56:908–943
18. Decaens T, Roudot-Thoraval F, Bresson-Hadni S, et al. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. Liver Transpl 2005;11:767-75.
19. Decaens T, Roudot-Thoraval F, Bresson-Hadni S, et al. Role of immunosuppression and tumor differentiation in predicting recurrence after liver transplantation for hepatocellular carcinoma: A multicenter study of 412 patients. World J Gastroenterol 2006;12:7319-25.
20. Decaens T, Roudot-Thoraval F, Hadni-Bresson S, et al. Impact of UCSF criteria according to pre- and post-OLT tumor features: Analysis of 479 patients listed for HCC with a short waiting time. Liver Transpl 2006;12:1761-9
21. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *JASA* 1999;94:496-509
22. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157-72; discussion 207-12.
23. Dutkowski P, Linecker M, DeOliveira ML, Müllhaupt B, Clavien PA. Gastroenterology. 2015;148: 307-23
24. NHS blood and transplant liver advisory group.
Liver transplantation for HCC in the UK - Report from a National Consensus Meeting (Birmingham, January 2014): LT for HCC-Consensus meeting summary. LAG(14)9b. Online access:
http://www.odt.nhs.uk/pdf/advisory_group_papers/LAG/HCC_recommendations_IR_TS_b_NAS_Work_in_Progress.pdf

25. Piñero, F., Tisi Baña, M., de Ataide, E. C., Hoyos Duque, S., Marciano, S., Varón, A., Anders, M., Zerega, A., Menéndez, J., Zapata, R., Muñoz, L., Padilla Machaca, M., Soza, A., McCormack, L., Poniachik, J., Podestá, L. G., Gadano, A., Boin, I. S. F. F., Duvoux, C., Silva, M. and the Latin American Liver Research, Education and Awareness Network (LALREAN) (2016), Liver transplantation for hepatocellular carcinoma: evaluation of the alpha-fetoprotein model in a multicenter cohort from Latin America. Liver Int. doi:10.1111/liv.13159 2016 May 12.

26. Lee JW, Paeng JC, Kang KW, Kwon HW, Suh KS, Chung JK, Lee MC, Lee DS. Prediction of tumor recurrence by 18F-FDG PET in liver transplantation for hepatocellular carcinoma.J Nucl Med. 2009;50(5):682-7

27. 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(1):53-61

28. Hong G, Suh KS, Suh SW, Yoo T, Kim H, Park MS, Choi Y, Paeng JC, Yi NJ, Lee KW. Alpha-fetoprotein and (18)F-FDG positron emission tomography predict tumor recurrence better than Milan criteria in living donor liver transplantation. J Hepatol. 2016;64(4):852-9

Author names in bold designate shared co-first authorship

Legends of figures

Figure 1:

Risk of recurrence according to pre LT AFP thresholds as defined in the AFP model.

Figure 2:

Overall probabilities of recurrence and survival according to the AFP score cut off of 2 (A and B) or Milan criteria (C and D).

Figure 3:

Probabilities of death not related to HCC recurrence as assessed by competing risk analysis according to the AFP model.

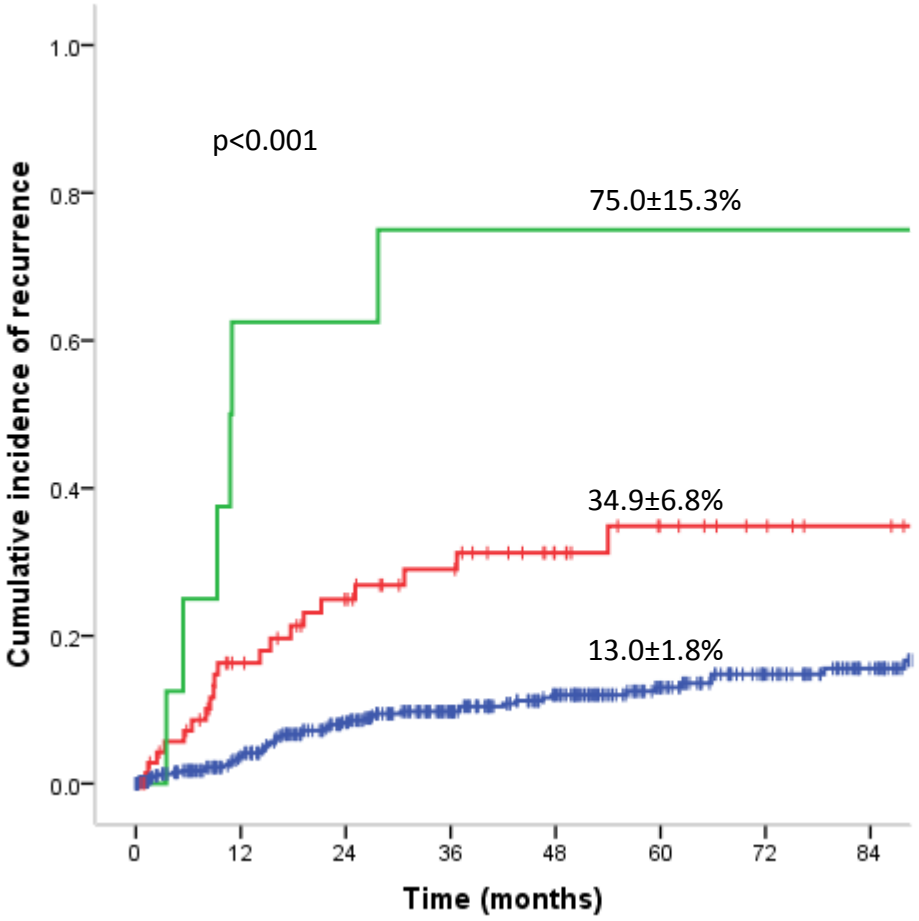
Figure 4:

Probabilities of recurrence according to the AFP score cut off of 2, in patients fulfilling or not Milan criteria.

Figure 5:

Probabilities of recurrence and survival according to the AFP score cut off of 2 in the HCV population (A and B) and in the HBV population (C and D).

Figure 1

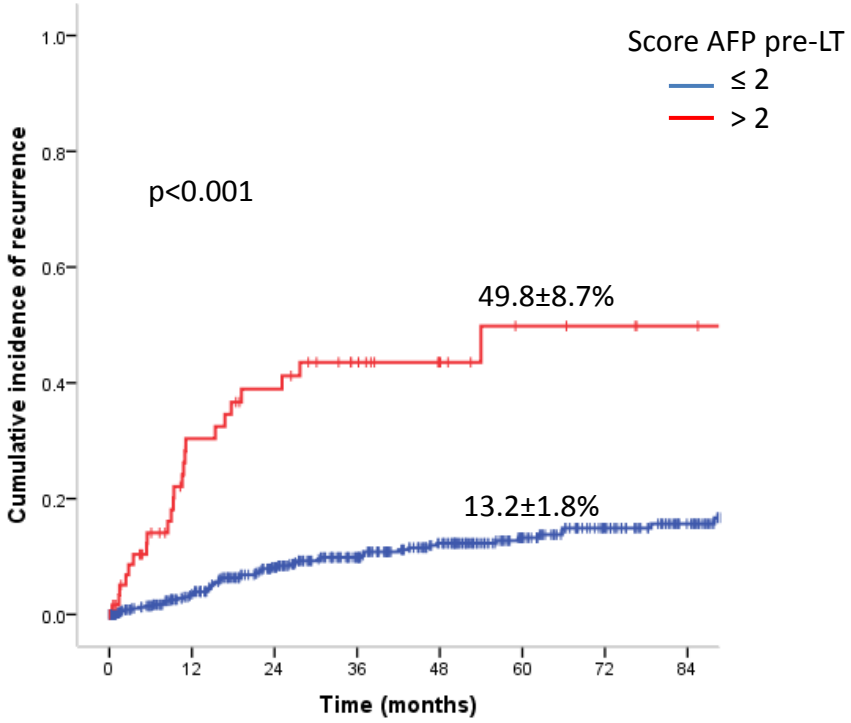


At risk

AFP ≤100	492	399	328	268	216	162	125	92
AFP]100-1000	73	51	41	33	22	15	11	8
AEP >1000	8	3	3	2	2	2	2	2

Figure 2

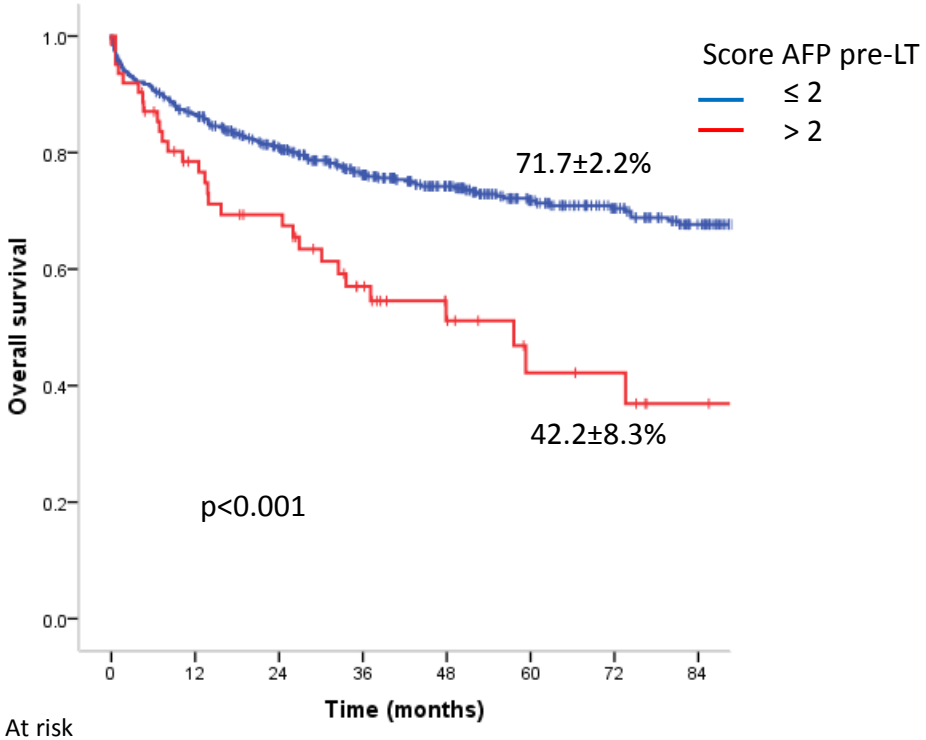
A



At risk

score≤2	512	420	345	284	228	172	132	98
Score ≥3	62	33	27	19	12	7	6	4

B

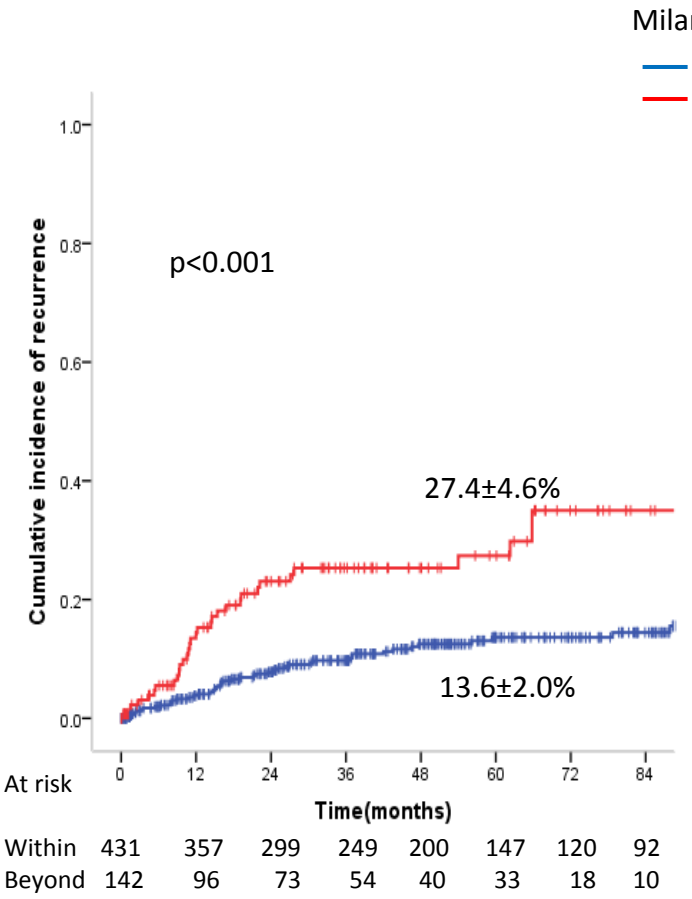


At risk

Score≤2	512	431	365	298	239	179	141	105
Score≥3	62	43	36	24	15	9	7	4

Figure 2

C


Milan criteria pre-LT
within
beyond
Cumulative incidence of recurrence
1.0
0.8
0.6
0.4
0.2
0.0
p<0.001
27.4±4.6%
13.6±2.0%
0
12
24
36
48
60
72
84
Time(months)
At risk
Within 431 357 299 249 200 147 120 92
Beyond 142 96 73 54 40 33 18 10

D

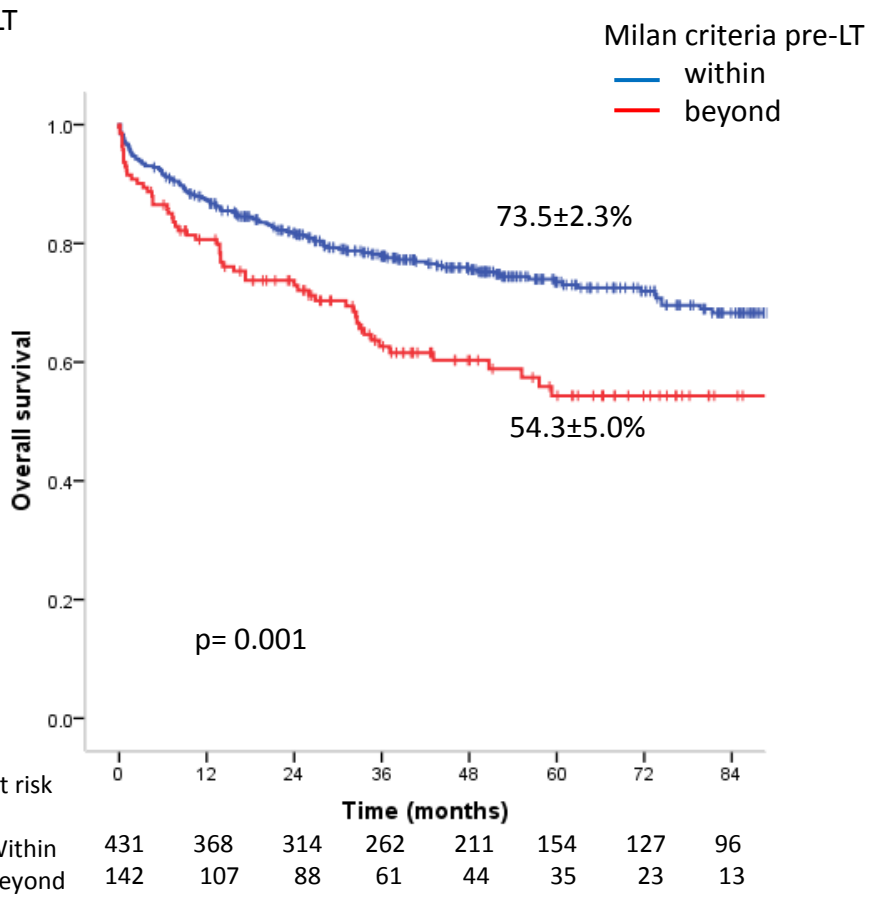
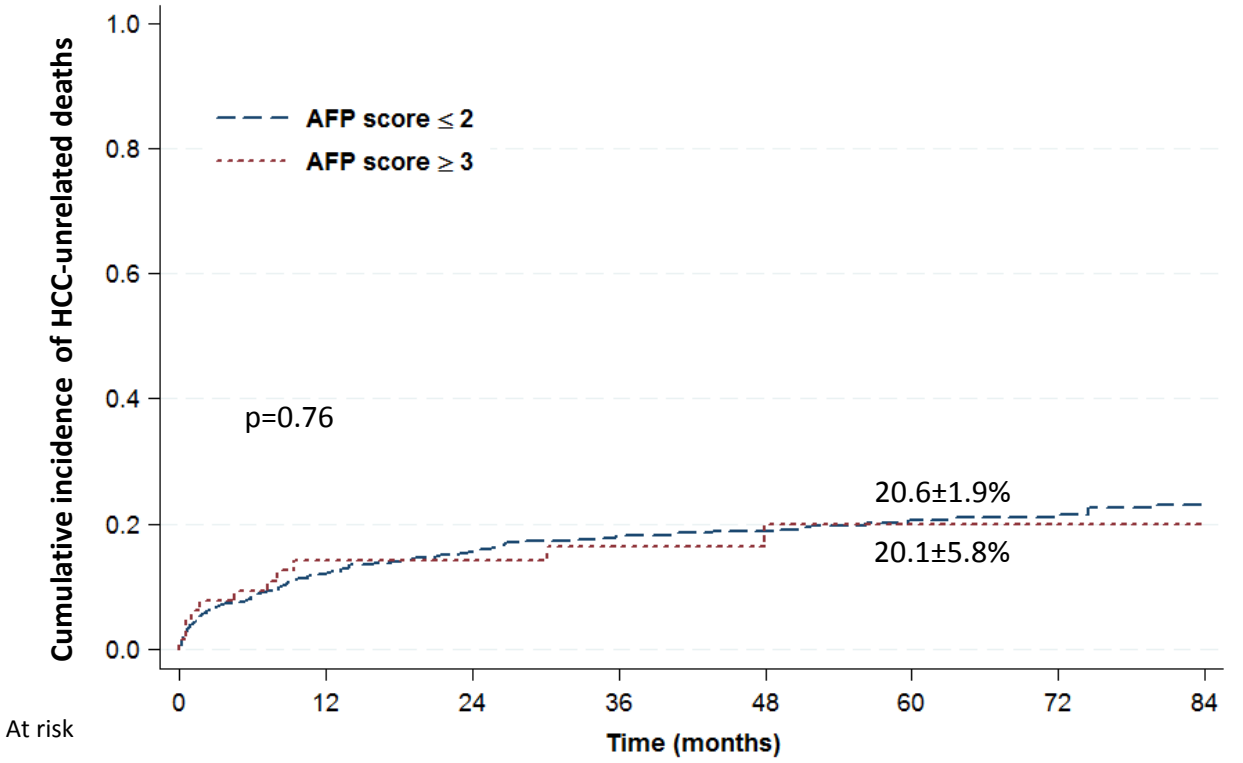

Milan criteria pre-LT
within
beyond
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
73.5±2.3%
54.3±5.0%
p= 0.001
0
12
24
36
48
60
72
84
Time (months)
At risk
Within 431 368 314 262 211 154 127 96
beyond 142 107 88 61 44 35 23 13

Figure 3

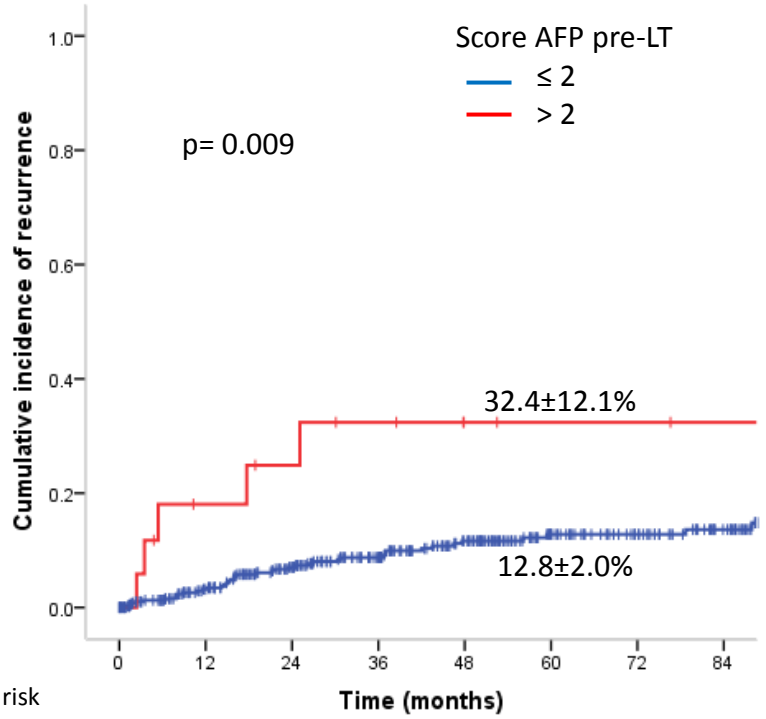


At risk

Score≤2	512	434	378	323	275	220	184	151
Score≥3	62	49	46	40	33	29	28	26

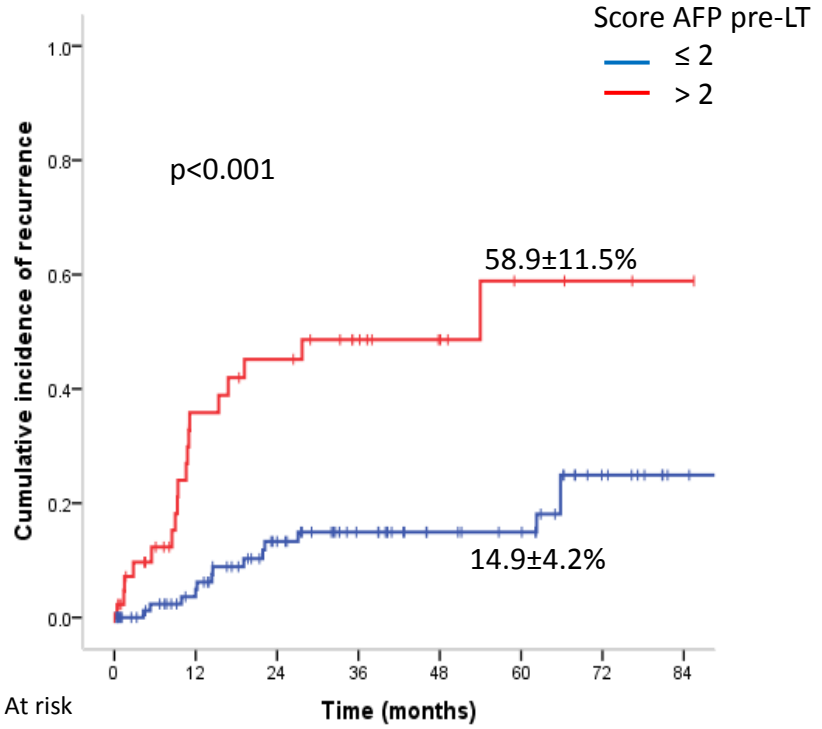
Figure 4

A. Patients within Milan criteria



At risk								
Score≤2	415	345	289	241	195	142	116	89
Score≥3	17	12	10	8	5	4	4	3

B. Patients beyond Milan criteria

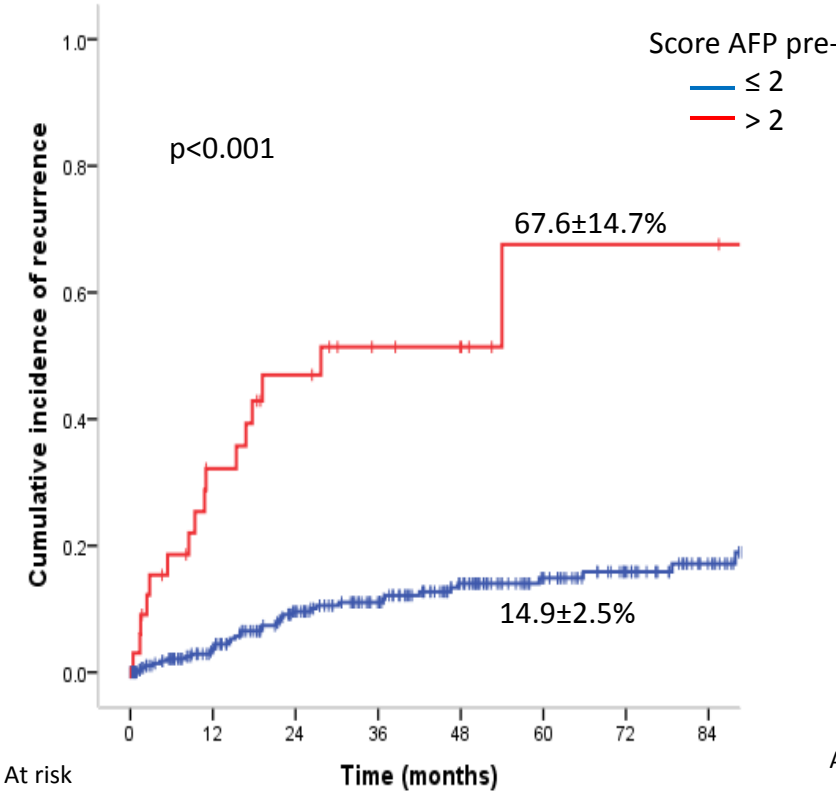


At risk									At risk
Score≤2	97	75	56	43	33	30	16	9	Score≤2
Score≥3	45	21	17	11	7	3	2	1	Score≥3

Figure 5

HCV population

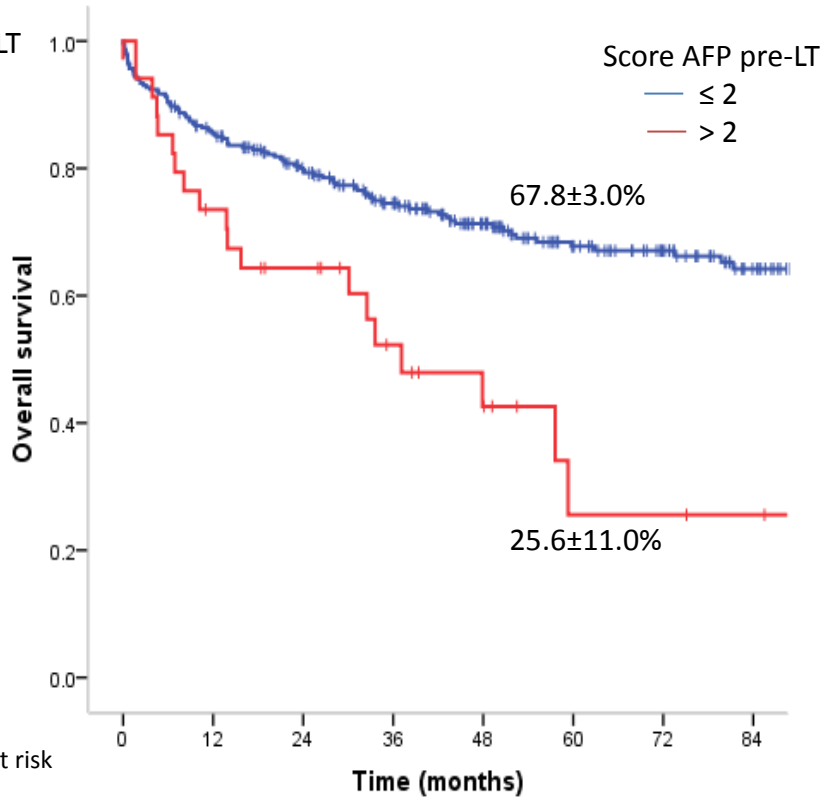
A- recurrence



At risk

Score≤2	303	245	198	166	130	97	77	54
Score≥3	34	19	13	8	6	2	2	2

B-Overall survival

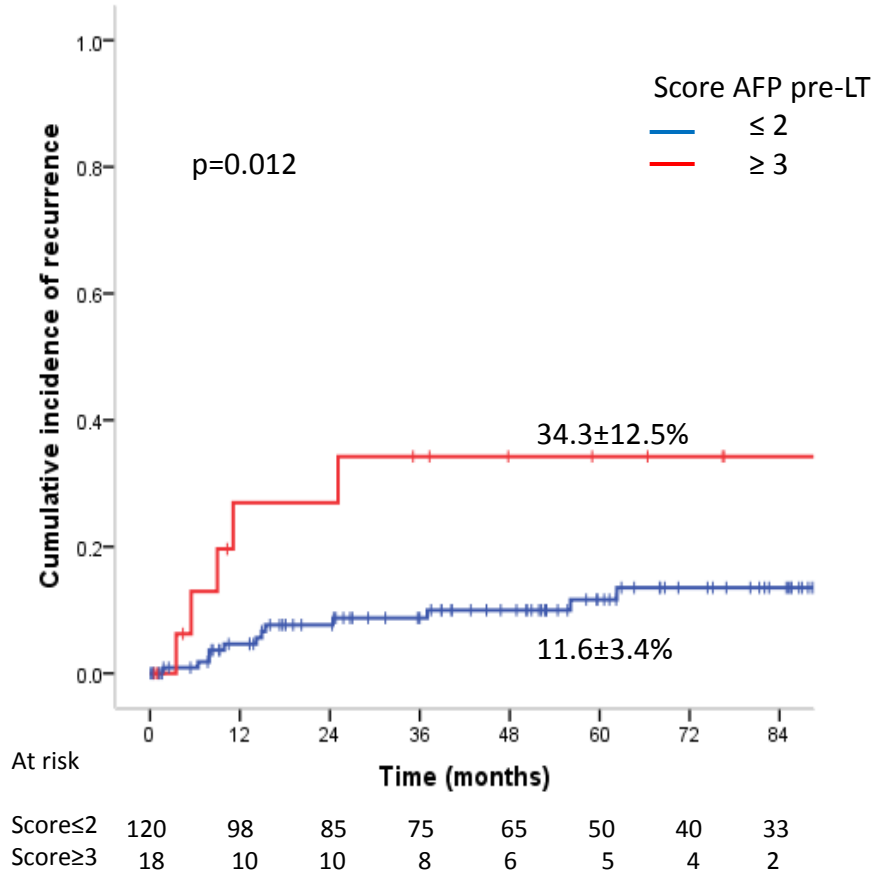


At risk

Score≤2	302	252	215	176	137	102	83	58
Score≥3	34	24	19	12	8	3	4	2

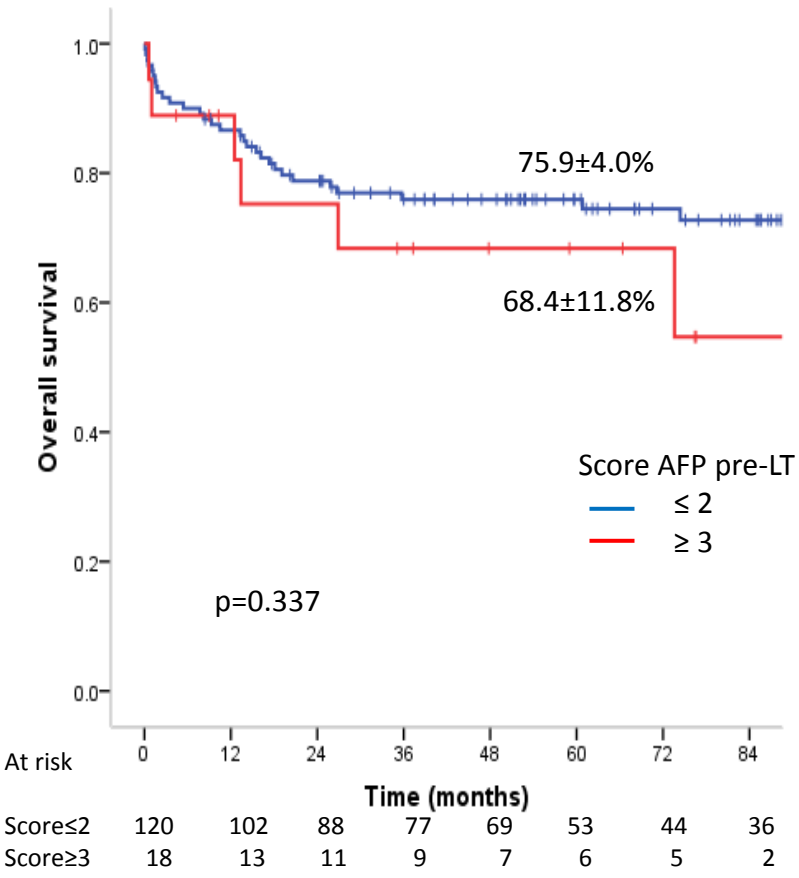
Figure 5
HBV population

C-Recurrence



At risk								
Score≤2	120	98	85	75	65	50	40	33
Score≥3	18	10	10	8	6	5	4	2

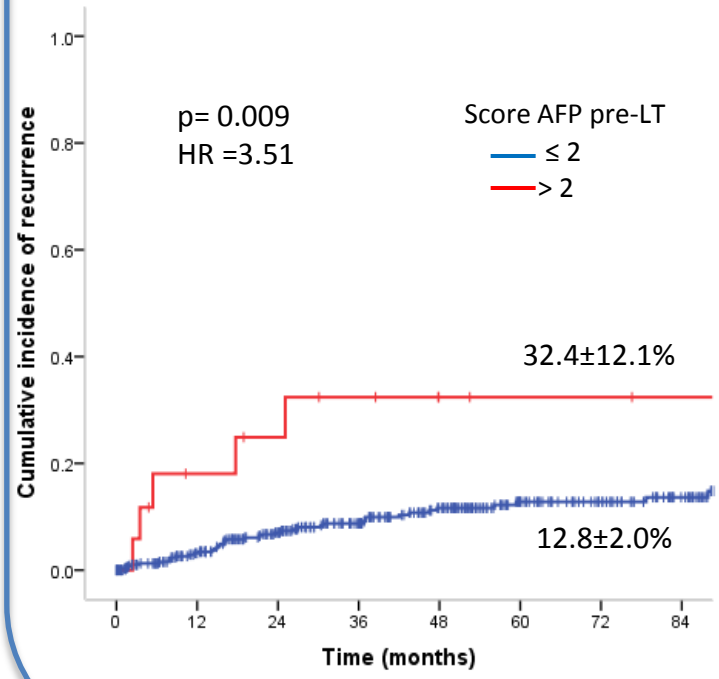
D-Overall survival



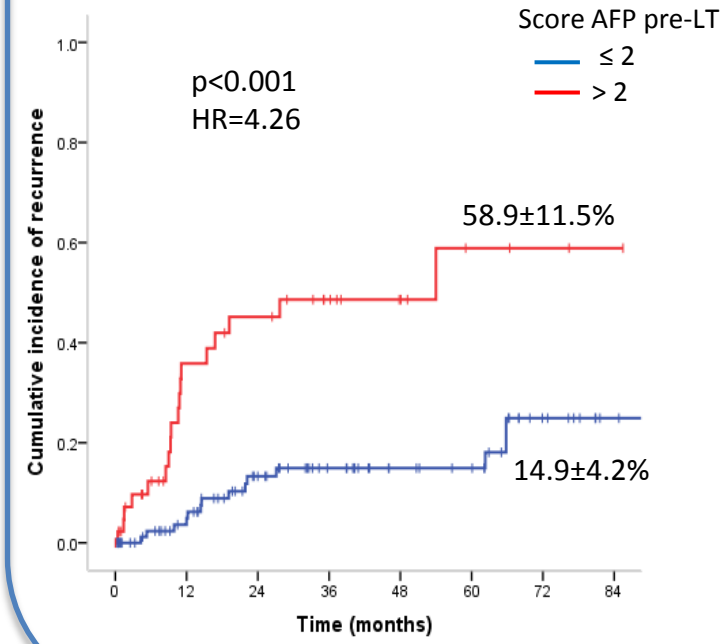
At risk								
Score≤2	120	102	88	77	69	53	44	36
Score≥3	18	13	11	9	7	6	5	2

Liver transplantation for hepato-cellular carcinoma in a cohort of 574 patients with a majority of post-hepatitic C and B cirrhosis : the AFP score predicts recurrence better than Milan criteria. The AFP score can be proposed for adequate selection of HCC candidates in programs with a high proportion of viral/HCV-related cirrhosis.

A. Patients within Milan criteria



B. Patients beyond Milan criteria



Tables

Table 1: Simplified, user-friendly version of the AFP model.

The score is calculated by adding the individual points for each obtained variable.

A cutoff of 2 separates between patients at high and low risk of recurrence. In this simplified version, a cut-off of 2 selected exactly the same patients as the original Cox score 0.7 cut-off.

Variables	β coefficient	Hazard ratio	Points
Largest diameter			
≤ 3 cm	0	1	0
3 - 6 cm	0.272	1.31	1
> 6 cm	1.347	3.84	4
Number of nodules			
1-3	0	1	0
4 and more	0.696	2.01	2
AFP level (ng/mL)			
≤ 100	0	1	0
]100-1000]	0.668	1.95	2
> 1000	0.945	2.57	3

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Table 2: Baseline characteristics of the study population

Males (n,%)	497 (86.6)
Age at listing/at LT(yrs)	55.8±7.5/56.9±7.6
MELD score (median, [IQR])	**12 [10-16]**
Child-Pugh (A/B/C) (n,%)	196 (34.1%)/268(46.7%)/110 (19.2%)
Causes of liver disease (HCV/HBV/Alcohol/others)	**387(58.7%)/138 (24%)/**67 (11.7%)/32 (5.6%)
Number of nodules (median,[IQR]), (range)	2 [1-2], (1-8)
Max Diameter (cm) (median,[IQR], (range)	2.5 [2-3.5], (1-21)
AFP (ng/mL) at listing (median,[IQR], (range)	9 [3.9-30.1], (0.4-17500)
AFP (ng/mL) at last evaluation	10.4 [4.3-33.3], (0.5-22455)
Milan criteria [in/out, (%)]	**432/142 (75.3% vs 24.7%)**
AFP score: ≤ 2 vs > 2	**512/62 (89.2% vs 10.8%)**
Median waiting time (months)	8.6 [3.6-16.0]
Bridging therapies (n,%)	486 (84.7)
Post-operative deaths (n,%)	44 (7.7)
Overall Recurrence rate (n,%)	81 (13.5)
Follow-up (months) (median, [IQR])	40.9 [18.4-73.6]

Table 3: Explant-based comparison of pathological features of HCC according to the AFP model.

	AFP score ≤ 2 n=512	AFP score > 2 n=62	p
Macro-vascular invasion [n, (%)]	16 (3.2)	5(8.3)	0.051
Micro-vascular invasion [n, (%)]	96 (19.4)	27(45.0)	<0.001
Poorly differentiated tumour [n,(%)]	116 (32.2)	28 (51.9)	0.009
Number of nodules (med, [IQ])	2 [1-3]	3 [1-5]	0.001
Diameter of nodules (med, [IQ])	2.5 [1.8-3.5]	4.5 [2.5-6]	<0.001