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CANCER

Liver transplantation for hepatocellular carcinoma: evaluation of the alpha-fetoprotein model in a multicenter cohort from Latin America

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Abstract

Background & Aims: The French alpha-fetoprotein (AFP) model has recently shown superior results compared to Milan criteria (MC) for prediction of hepatocellular carcinoma (HCC) recurrence after liver transplantation (LT) in European populations. The aim of this study was to explore the predictive capacity of the AFP model for HCC recurrence in a Latin-American cohort. *Methods:* Three hundred twenty-seven patients with HCC were included from a total of 2018 patients transplanted at 15 centres. Serum AFP and imaging data were both recorded at listing. Predictability was assessed by the Net Reclassification Improvement (NRI) method. *Results:* Overall, 82 and 79% of the patients were within MC and the AFP model respectively. NRI showed a superior predictability of the AFP model against MC. Patients with an AFP score >2 points had higher risk of recurrence at 5 years Hazard Ratio (HR) of 3.15 ($P = 0.0001$) and lower patient survival (HR = 1.51; $P = 0.03$). Among patients exceeding MC, a score ≤2 points identified a subgroup of patients with lower recurrence (5% vs 42%;

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Abbreviations

AFP, alpha-fetoprotein; CI, confidence interval; CT, computerized tomography; HCC, hepatocellular carcinoma; HR, hazard ratio; HV, high volume centre; IQR, interquartile range; LT, liver transplantation; LV, low volume centre; MC, milan criteria; MELD, model for end-stage liver disease; MRI, magnetic resonance imaging; NAFL, non-alcoholic fatty liver; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TAC, trans-arterial chemoembolization; TTD, total tumour diameter; TTR, time to recurrence; WL, waiting list.

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$P = 0.013$) and higher survival rates (84% vs 45%; $P = 0.038$). In cases treated with bridging procedures, following restaging, a score >2 points identified a higher recurrence (HR 2.2, $P = 0.12$) and lower survival rate (HR 2.25, $P = 0.03$). A comparative analysis between HBV and non-HBV patients showed that the AFP model performed better in non-HBV patients. *Conclusions:* The AFP model could be useful in Latin-American countries to better select patients for LT in subgroups presenting with extended criteria. However, particular attention should be focused on patients with HBV.

Keywords
alpha-fetoprotein – candidate selection – liver cancer – prediction

Key points

- This is the first non-European, multicenter cohort to explore the predictive capacity of the AFP model to improve selection of patients with HCC for LT.
- The AFP model discriminated better between patients with low and high risk of recurrence.
- Among patients exceeding MC, cut-off values of ≤2 points in the AFP model further identified a subgroup of patients with low risk of recurrence.
- The AFP model performed better in non-HBV patients.

Rates of recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) have been steadily declining since Milan criteria (MC) were introduced to improve transplant candidate selection (1, 2). Moreover, organ allocation priority policies have been implemented for patients within MC since the application of the Model for End Stage Liver Disease (MELD) (3). However, despite application of these criteria, HCC recurrence still occurs in 10–15% of the patients (4–6). Conversely, other studies have challenged MC restriction enforcement, as excellent outcomes have been shown in selected group of patients exceeding these criteria (7, 8).

More recently, increasing focus is shifting towards using biological tumour behaviour as a selection tool, including pretransplant serum alpha-fetoprotein (AFP) (9–12). Elevated AFP prior to LT has been associated with higher recurrence rates (9–13). The AFP model, which adds use of pre-LT imaging to serum AFP to predict HCC recurrence after LT, has been shown to be superior to Milan criteria, both in French and Italian populations (14, 15). The model is based on a scoring system (0–9 points), which assigns values to: largest tumour diameter, number of HCC nodules as well as pre-LT AFP levels. A cut-off value of two points identifies patients with excellent survival and lower recurrence rate at 5 years (14).

Transferability of the AFP scoring system to other populations will help validate the true HCC recurrence predictive capacity of this model. In French cohorts, alcoholic cirrhosis followed by hepatitis C (HCV) were the most frequent causes of HCC, and in the Italian cohort the AFP model performed better for HCV than hepatitis B (HBV) patients. It is in these different country-specific scenarios where the new prediction models need to be evaluated further. To the best of our knowledge, no external evaluation of the AFP model in a non-European cohort has been reported to date. Our objective therefore was to test the AFP model accuracy for prediction of HCC recurrence after LT, compared to Milan criteria, in a multicenter cohort from Latin America.

Patients and methods

Participating centres

This study was conducted analysing a multicenter Latin-American cohort of consecutive adult liver transplant patients (>17 years of age) between June 1 2005 and December 31 2011 from 15 different LT centres in the region. Participating centres appointed a study coordinator responsible for data collection. In cases of conflicting or missing data, central revision and resubmission were requested.

Cohort characteristics and data collection

Criteria for inclusion required patients to be adult cirrhotic or non-cirrhotic LT recipients with confirmed HCC in the explanted liver. Patients were excluded if: (i) Incidental HCC was found in explanted liver pathology, without a preceding diagnoses on imaging (iHCC); or (ii) if venous or extrahepatic tumour involvement was found on pre-LT images.

Pretransplant recipient and tumour characteristics including serum AFP levels and cross-sectional imaging records were both evaluated at time of listing (16). Preselected cut-off values for pre-LT serum AFP were those selected by Duvoux *et al.* (14). Tumours were classified according to Milan and AFP model criteria, depending on size and number of lesions detected on pre-LT computed tomography (CT) or magnetic resonance images (MRI). French AFP scores (0–9 points) were calculated depending on: largest tumour diameter (≤3 cm = 0 points, 3–6 cm = 1 point, >6 cm = 4 points), number of HCC nodules (1–3 nodules = 0 points, ≥4 nodules = 2 points) and pre-LT AFP levels ng/ml (≤100 = 0 points, 101–1000 = 2 points and >1000 = 3 points) (14). Standard patient selection in all centres was limited to patients with tumours meeting MC. Transplantation for patients exceeding MC was discussed at each transplant centre on a case-by-case basis. Site-specific organ allocation policies were also registered.

Tumour treatment before LT was reviewed, namely: trans-arterial chemoembolization (TACE), radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) and liver resection. Among patients who received any local/regional tumour treatment prior to transplant, both last serum AFP and imaging results considered for restaging were also registered. In patients exceeding MC who were treated before LT, downstaging was defined as reducing the tumour size specifically to meet MC.

Explanted liver data collected included: macroscopic and microscopic evaluation of each nodule, number and diameter (cm) of each, presence of microvascular invasion and degree of tumour differentiation according to pathological standards and Edmonson Steiner grading system (17). Nodules of largest diameter were identified as the major nodule. Necrotic nodules were also measured including necrotic and viable tumour diameter.

Primary endpoints analysed were as follows: 5-year patient survival and HCC recurrence. All patients were followed until death or last ambulatory visit. Post-transplant follow-up for HCC recurrence consisted of one CT or MRI, bone scintigraphy and serum AFP assay every 6 months, as recommended (18). Recurrence was determined on the basis of imaging criteria plus serum AFP or by biopsy. Time to recurrence (TTR) was considered a robust clinical outcome measure and calculated as the time in months elapsed between transplantation and diagnosis of recurrence. Early recurrences were defined as those occurring during the first 12 months of LT (19).

To better compare results between transplant centres, LT centres were ranked as High Volume (HV) or Low Volume (LV) if more or less than 30 procedures were performed annually.

All procedures followed were in accordance with STROBE guidelines (20). This study was approved by the Austral University Faculty of Medicine and by each centre ethics committee; complied with the ethical standards (institutional and national) and with Helsinki Declaration of 1975, as revised in 2008. Patient consent was obtained in all subjects included.

Statistical analysis

Categorical data were compared using Fisher's exact test (two tailed) or Chi-Square (χ^2) test. Continuous variables were compared with Student's *T* test or Mann–Whitney *U* test according to their distribution respectively. Hazard ratios (HR) for HCC recurrence were calculated using Cox regression multivariate analysis (95% CI) including variables evaluated by Duvoux *et al.* (14). Variables with a *P* value <0.05 after the univariate analysis were included in the multivariate Cox model, generated by stepwise backward elimination (Wald test). Calibration and validation of the model were performed using the Hosmer-Lemeshow test and bootstrapping technique (1000 samples) respectively. Proportional hazard assumptions were assessed by Schoenfel test and by log-minus log survival curves. Kaplan–Meier survival curves were compared using the log-rank test (Mantel-Cox). For TTR only recurrences were censored, excluding deaths as a composite endpoint. Moreover, a competing risk analysis to evaluate the outcome against the incidence of non-HCC related death was also performed.

To compare AFP score predictability to MC, net reclassification improvement (NRI) and recurrence rates for patients within and beyond MC were estimated according to the new model. NRI focus on reclassification tables constructed separately for patients with and without events, and quantifies the correct movement in separated categories (upwards for events and downwards for non-events). Any 'upwards' movement in an event category implies improved classification for the subject, and any 'downwards' movement indicates worse classification. NRI does not provide net proportions; maximum value of the net reclassification index is 2. Collected data were analysed with SPSS V20.0 (SPSS Inc., Chicago, IL, USA) and STATA 10.0.

Table 1. Patients' baseline characteristics

Variable	Values
Age, years (±SD)	57 ± 8
Gender, male, *n* (%)	267 (81.7)
Median time on waiting list, (IQR), months	7.0 (1.0–9.0)
MELD, (±SD)*	16.7 ± 7.9
Supplementary MELD points, *n* (%)	185 (56.6)
Non-cirrhotic liver, *n* (%)	6 (1.8)
Child Pugh A/B/C, *n* (%)	114 (35.5)/ 130 (40.5)/77 (24.0)
Aetiology of liver disease, *n* (%)	
Hepatitis B virus	94 (28.7)
Hepatitis C virus	89 (27.2)
Hepatitis B and C virus	3 (0.9)
Alcohol	58 (17.7)
NASH	25 (7.6)
Cryptogenic	24 (7.3)
Cholestatic†	13 (3.9)
Autoimmune	9 (2.7)
Hemochromatosis	6 (1.8)
Miscellaneous	9 (2.7)
Living donor, *n* (%)	3 (0.9)
Within Milan at listing, *n* (%)	269 (82.3)
Tumour number, (±SD)	1.5 ± 0.9
Major nodule diameter, cm, (±SD)	3.3 ± 1.3
AFP at listing, ng/ml, median (IQR)	14.4 (4.6–96.0)
Tumour biopsy before LT, *n* (%)	5 (1.5)

MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis.

*Laboratory MELD score before liver transplant.

†Cholestatic: primary biliary cholangitis, primary and secondary sclerosing cholangitis.

Results

Patients and tumour characteristics

From a total of 2018 consecutive adult LT patients, operated at 15 different centres during the study period previously described, 422 patients had HCC, of which 327 were included in this study. Among patients excluded, 77 presented incidental HCC and in 18 patients HCC was not confirmed in the explanted liver: 15 showed regenerative nodules, 1 had biliary hamarthoma and 2 patients had cholangiocarcinoma. Percentage of patients contributed by participating LT centres per country was as follows: five transplant programmes from Argentina (n = 98, 30.0%), on from Brazil (n = 90, 27.5%), two from Colombia (n = 65, 19.9%), four from Chile (n = 49, 15.0%) and 1 LT centre from Peru (n = 11, 3.4%), Uruguay (n = 9, 2.8%) and Mexico (n = 5, 1.5%).

Overall, 56.6% (n = 185) of the patients within MC were assigned supplementary MELD points. Patients within MC from Argentina, Brazil, Uruguay, Peru and Chile could receive additional MELD points while on the waiting list. Median time on waiting list was 7.2 months (IQR 1.0–9.0 months). Chronic hepatitis B virus infection (HBV) was the main cause of liver disease (Table 1). Bridging therapies prior to transplantation were performed in 47.4% of patients (n = 155). Before inclusion on the waiting list, 71 procedures were conducted in 63 patients including: 36 TACEs, 19 RFAs, 5 PEIs and 11 liver resections. While on the waiting list, 110 patients were treated (TACE n = 85, RFA n = 19, PEI n = 5 and liver resection n = 3). Downstaging from exceeding to meeting MC was attempted in 31 patients and successfully achieved in 13.

Explanted liver findings showed that 61.8% (n = 202) of the patients were within MC. Microvascular invasion was present in 23.8% (n = 77), and 22.9% (n = 75) had undifferentiated tumours. Overall, 75 patients (22.9%) and 30 patients (9.2%) presented partial or complete major nodule necrosis respectively; HCC was confirmed in all patients sampling viable foci. Patients with prior liver resection as bridging therapy all presented viable HCC in the explanted liver. Serum AFP levels >1000 ng/ml at listing were associated with more microvascular invasion, undifferentiated tumours (Supporting Information) and higher recurrence and lower survival rates (Fig. 1).

Drugs most used for initial immunosuppression were Tac (58.3%), MMF (69.6%) and steroids (98.7%). Corticosteroids use was tapered between the 3rd and the 24th month after LT from 85.7 to 18.4%. Three months after LT, 5.9% (n = 16) of patients were taking mTORs inhibitors, a figure that had increased to 19.8% (n = 42) by 2 years follow-up.

Milan criteria and French model assessment at listing

At listing, 82.3% (n = 269) and 79.1% (n = 257) of the patients were within MC and the AFP model respectively. Cut-off values described by Duvoux *et al.* showed

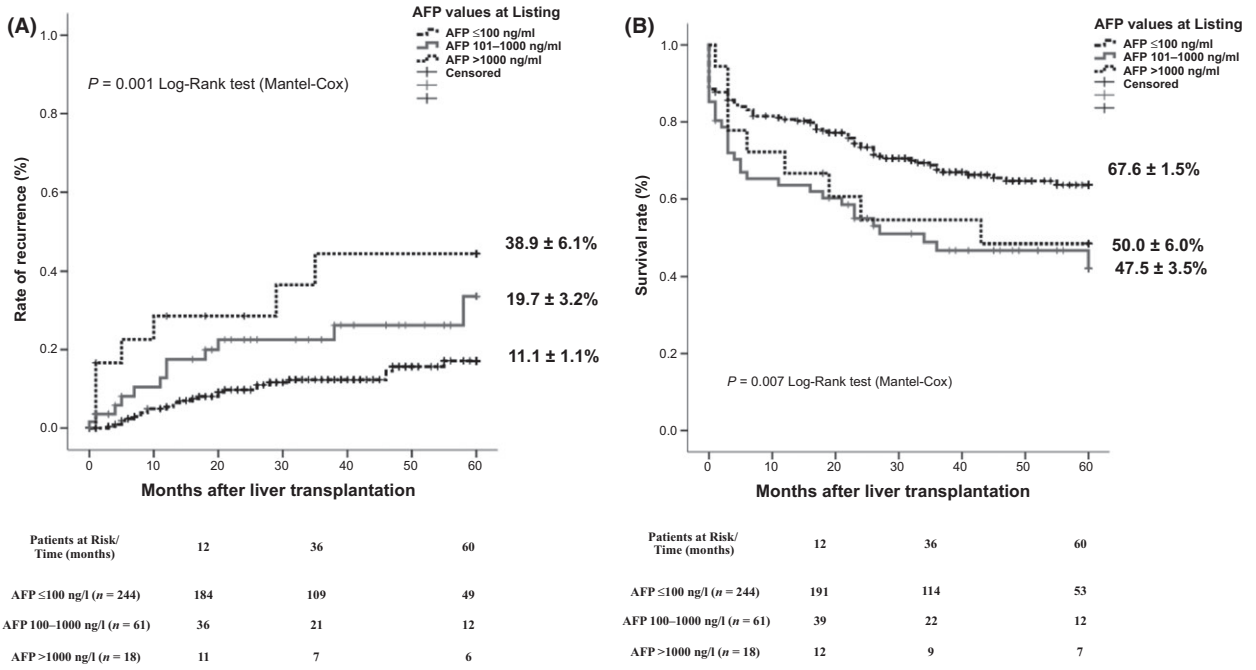

Fig. 1. Tumour recurrence (Panel A) and overall patient survival (Panel B) according to α-fetoprotein level (AFP) at time of listing. Kaplan–Meier curves (log Rank test).

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74.6% of the patients had a serum AFP ≤100 ng/ml (n = 244), 18.7% an AFP value between 101 and 1000 ng/ml (n = 61) and 5.5% >1000 ng/ml (n = 18). Four patients lacked AFP values at time of listing; two were nevertheless included in the analysis because imaging data scores alone, as specified in the French model, were >2 points. Among patients within MC, 89 and 11% had AFP scores ≤2 points and >2 points; whereas in patients exceeding MC, 33.3 and 66.7% had AFP scores ≤2 and >2 points respectively.

Survival and recurrence analysis

Outcomes were assessed in all patients during follow-up (median 45.0 months; IQR 30.0–66.0 months). Overall patient survival and recurrence rates at 1, 3 and 5 years were 76.8, 64.8 and 62.7% (n = 122 deaths) and 7.3, 12.8 and 15.0% (n = 49 recurrences) respectively. Main cause of death was recurrent HCC (n = 39), followed by sepsis (n = 30). Median TTR was 13.0 months (IQR 6.0–28.0 months), with early recurrences occurring in 24 patients (48.9%). Median survival following recurrence was 12.0 months (IQR 5.0–26.0 months). Cox regression analysis of pretransplant risk factors for 5-year HCC recurrence is shown on Table 2, panels A–B.

Low volume centres (n = 6) had higher proportion of patients exceeding MC at listing compared to HV (31.6% vs 15.9%; P = 0.02), and more cases with French scores >2 points (26.3% vs 19.9%; P = 0.23). Although no significant difference in 5-year survival rates was observed, higher recurrence rate was seen in LV (23.7% vs 13.8%; HR 1.73, CI 0.84–3.57; P = 0.13).

Impact on liver transplant candidates of the French model compared to Milan criteria

Table 3 shows a comparative analysis between patients with AFP model scores >2 points and ≤2 points. The AFP cut-off accurately discriminated patients with higher risk of recurrence at 5 years 30.1% vs 10.1% with a HR of 3.15 (CI 1.77–5.61; P = 0.0001). In addition, patients with a score >2 points had lower 5-year survival rates compared to patients with ≤2 points: 48.5% vs 66.1%, HR of 1.51 (CI 1.02–2.23; P = 0.03) (Fig. 2, Panel A–D).

Among patients exceeding MC, 5-year recurrence rate was higher in patients with AFP model score >2 points compared to those with ≤2 points (42.1% vs 5.3%; P = 0.013). In addition, 5-year survival benefit was observed in patients exceeding MC with AFP model score ≤2 points compared to patients with >2 points 84.2% vs 44.7% (HR 8.38, CI 1.11–63.49; P = 0.038). Considering patients within MC, higher 5-year tumour recurrence rate and lower patient survival were observed in patients with AFP score >2 points compared to patients with ≤2 points 16.7% vs 10.5% (HR 1.68, CI 0.64–4.41; P = 0.32) (Fig. 3, Panels A–D), although the difference was not statistically significant. Finally, there was not a statistical significant difference considering 5-year recurrence (11.5% vs 5.3%; P = 0.41) and survival (63.6% vs 84.2%; P = 0.13) between patients within MC and those exceeding MC with an AFP score ≤2 points (n = 19).

Table 2. Panel (A) Variables associated with 5-year HCC recurrence after liver transplantation. Univariate Cox regression. Panel (B) Multivariate Cox regression analysis of pretransplant risk factors associated with 5-year hepatocellular carcinoma Recurrence and Bootstrapped bias corrected – confidence intervals

Panel (A)

Variable	5-year incidence of recurrence, (%)	Hazard ratio (95% CI)	P
WL time <3 months			
Yes (n = 203)	14.3	0.98 (0.55–1.77)	0.97
No (n = 121)	14.9		
Underlying liver disease			
HCV (n = 92)	16.3	1.03 (0.56–1.89)	0.91
HBV (n = 94)	13.8	1.06 (0.56–2.01)	0.84
Treatment before LT			
Yes (n = 155)	19.4	1.74 (0.98–3.09)	0.08
No (n = 172)	11.0		
Milan criteria*			
Within (n = 269)	11.5	0.36 (0.21–0.66)	0.001
Exceeding (n = 58)	31.0		
Number of tumours*			
1–3 nodules (n = 314)	13.4	4.60 (3.06–10.28)	0.0001
≥4 nodules (n = 13)	53.8		
Diameter of the largest tumour, cm*			
≤3 (n = 173)	10.4	–	–
3–6 (n = 132)	16.7	1.44 (0.77–2.69)	0.24
>6 (n = 22)	40.9	4.10 (1.84–9.13)	0.001
AFP level at listing, ng/ml			
≤100 (n = 244)	11.1	–	–
101–1000 (n = 61)	19.7	2.20 (1.11–4.34)	0.023
>1000 (n = 18)	38.9	3.66 (1.59–8.42)	0.002

Panel (B)

Variable	β	HR (CI 95%)	Bootstrapping CI 95%	P
Number of tumours				
1–3 nodules				
≥4 nodules	0.614	1.85 (1.19–2.87)	1.20–2.66	0.006
Largest diameter, cm				
≤3				
3–6	0.047	1.04 (0.54–2.04)	0.59–2.01	0.88
>6	1.330	3.78 (1.64–8.72)	1.67–7.90	0.002
AFP level at listing, ng/ml				
≤100				
101–1000	0.824	2.28 (1.10–4.71)	1.14–4.37	0.026
>1000	1.114	3.05 (1.32–7.04)	1.48–6.14	0.009

Normal values: alpha-fetoprotein 0.6–4.4 ng/ml.

AFP, alpha-fetoprotein; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; LT, liver transplantation; WL, waiting list.

*Assessed by imaging criteria. β-coefficient.

Table 3. Comparative analysis according to AFP model at listing

Variable	AFP score ≤2 *n* = 257 (79.1%)	AFP score >2 *n* = 68 (20.9%)	*P*
Age, years (±SD)	57 ± 8	54 ± 11	0.12
Waiting list, months, median (IQR)	4.0 (1.0–10.0)	2.0 (0.0–7.2)	0.12
MELD, (±SD)	16 ± 8	17 ± 8	0.68
Pretransplant images			
Within Milan, *n* (%)	238 (92.6)	30 (44.1)	0.0001
AFP, ng/ml, median (IQR)	10.7 (4.0–28.9)	173.0 (41.7–1082.5)	0.0001
AFP ≤100 ng/ml, *n* (%)	223 (86.8)	21 (31.8)	0.0001
AFP 100–1000 ng/ml, *n* (%)	34 (13.2)	27 (40.9)	0.0001
AFP >1000 ng/ml, *n* (%)	0 (0)	18 (27.3)	0.0001
Treatment during waiting list, *n* (%)	111 (43.2)	43 (63.2)	0.002
Explanted liver features			
Within Milan, *n* (%)	175 (68.1)	26 (38.2)	0.0001
Within up to 7, *n* (%)	223 (86.8)	42 (61.8)	0.0001
Macrovascular invasion, *n* (%)	3 (1.4)	6 (11.5)	0.002
Microvascular invasion, *n* (%)	43 (16.9)	32 (47.8)	0.0001
Nuclear grade >II, *n* (%)	47 (22.4)	28 (45.2)	0.001

Normal values: alpha-fetoprotein 0.6–4.4 ng/ml.
AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

A competing risk analysis-evaluating outcome against incidence of non-HCC–related death showed no significant differences between groups. Net reclassification improvement estimated between MC and the AFP model was 0.06 ($P = 0.01$), indicating that the overall HCC recurrence prediction was better with the French model (Supporting Information).

Impact of the AFP model in patients receiving local bridging therapies prior to transplantation

With respect to patients receiving local/regional treatments ($n = 155$), 90 patients were restaged after bridging therapies. At listing 73.3% ($n = 66$) of these patients were within MC and 70.8% ($n = 63$) within the AFP model criteria. At final evaluation, 78.9% ($n = 71$) and 76.4% ($n = 68$) of the patients were within Milan and AFP models respectively. Median time elapsed between last imaging evaluation and transplant was 1.0 month (IQR 0.0–4.0 months). After restaging, patients with a score >2 points ($n = 22$) had higher 5-year recurrence (HR 2.2, CI 0.77–6.12; $P = 0.12$) and lower survival rates (HR 2.25, CI 1.07–4.71; $P = 0.03$) compared to those with ≤2 points ($n = 68$) (Supporting Information).

Among patients attempted to downstaging, 64.5% ($n = 20$) had an AFP score >2 points at listing. After tumour restaging, 13 patients were within MC (41.9%) and 18 patients had scores ≤2 points (58.1%), with corresponding 5-year tumour recurrence rates of 23.1 and 16.7% respectively. Patients with AFP score >2 points ($n = 13$) had higher 5-year recurrence (HR 3.05, CI 0.73–12.78; $P = 0.11$) and lower survival rates (HR 3.30, CI 1.12–9.66; $P = 0.03$) compared to those with ≤2 points.

The AFP model and better performance in non-hepatitis B patients

Table 4 shows a comparative analysis between HBV ($n = 94$) and non-HBV patients ($n = 233$). Interestingly, the AFP model performed better for predicting HCC recurrence in non-HBV patients (Fig. 4, Panels A and B). The latter subgroup of patients showed higher 5-year tumour recurrence rates if AFP model scores were >2 points compared to patients with ≤2 points 8.8% vs 31.2% (HR 5.27, CI 1.91–14.55; $P = 0.001$); which was also the case for patients within ($n = 187$) and exceeding MC ($n = 46$), in whom 5-year recurrence rates were 8.8% vs 31.2% (HR 5.27, CI 1.91–14.54; $P = 0.001$) and 6.2% vs 50.0% (HR 8.62, CI 1.13–65.68; $P = 0.038$) if French scores were ≤2 or >2 points respectively.

Discussion

To the best of our knowledge, this is the first non-European, multicenter, Latin-American cohort study to explore the predictive capacity of the AFP model. Our results showed first, that in this cohort, just as in European populations, the AFP model discriminated better between patients with low and high risk of recurrence, resulting in a significant impact on patient survival. In contrast, although MC also distinguished two risk groups for HCC recurrence, 5-year survival rates did not differ, suggesting that the AFP model was better at identifying patients at higher risk of death after transplantation. Secondly, among patients exceeding MC, cut-off values of ≤2 points in the AFP model further identified a subgroup of patients with low risk of recurrence and 84% 5-year survival, thus rescuing patients beyond MC with excellent prognosis after

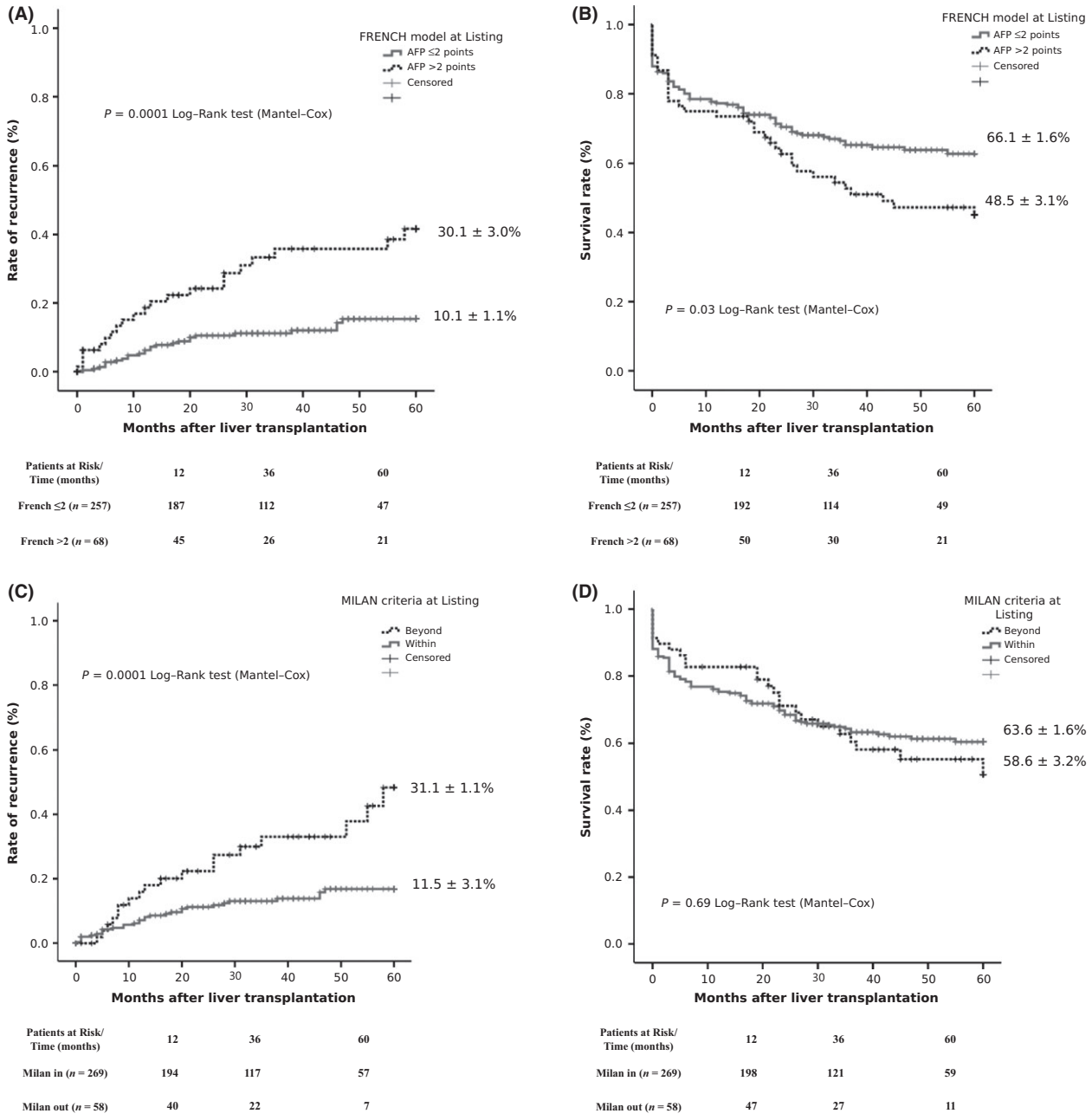

Fig. 2. Tumour recurrence and patient survival rates according to alpha-fetoprotein (AFP) model (Panels A–B) and Milan criteria (Panels C–D) at listing (Kaplan–Meier; log-rank test).

transplantation. A similar result was observed among patients within MC with an AFP score >2 points with a trend towards higher risk of recurrence and lower 5-year survival. Thirdly, when tumours were clinically reassessed after local/regional therapies, the AFP model still discriminated between patients with high and low risk of recurrence and improved or worse 5-year survival. Finally, the AFP model performed better in predicting HCC recurrence in non-HBV patients.

Efforts to modify LT selection criteria to optimize outcomes in patients with HCC should be considered, not only for the benefit of patients with HCC but also for justice and equipoise to prevail in allocation policies for non-HCC patients (21). When the AFP model was assessed in French (14) and Italian (15) cohorts, it proved to be superior to MC. In this study, a minority of patients falling within standard selection criteria exceeded the new AFP model. These patients showed

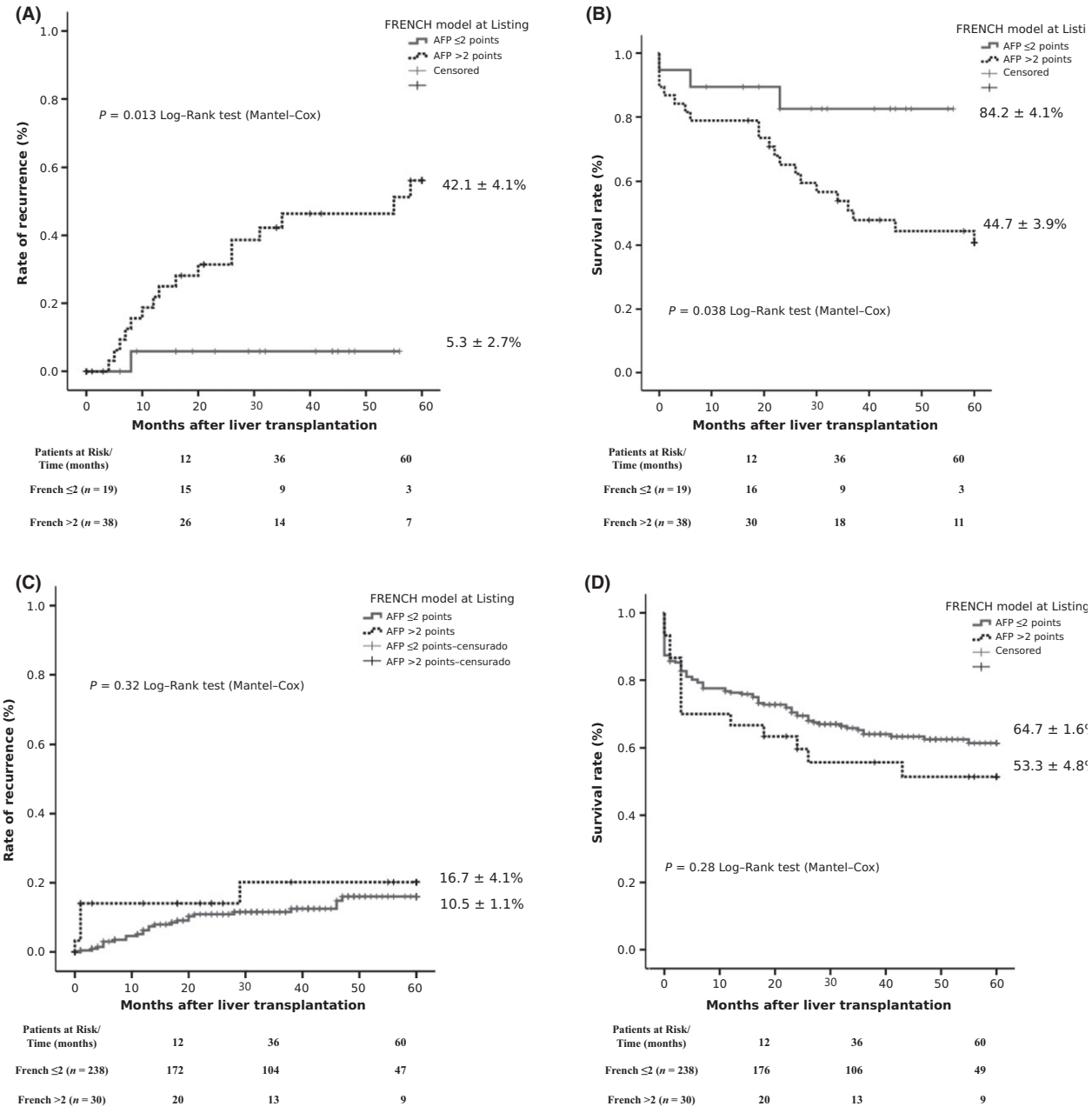

Fig. 3. Tumour recurrence and survival curves according to alpha-fetoprotein (AFP) model in patients exceeding (Panels A–B), or falling within Milan criteria at time of listing (Panels C–D). Tumour recurrence and survival curves according to AFP model in patients receiving local/regional bridging therapies before liver transplantation (Panels E–F). Kaplan–Meier; log-rank test.

higher recurrence rates compared to subjects with scores ≤2 points, although not to a statistically significant level, possibly because of the low number of patients included who were within Milan and had AFP model scores >2 points. Of note, the AFP model identified a subgroup of patients who exceeded MC, but still presented excellent outcomes.

Interestingly, this net benefit in survival may have been the result of incorporating AFP to the selection process (22–28). As originally reported by Duvoux *et al.*, in this study, elevated serum AFP was associated with higher recurrence and lower overall survival rates, and correlated with known pathological risk factors. The latter were significantly more prevalent in patients with AFP scores >2 points, suggesting presence of a more aggressive tumour. Although pre-LT serum AFP levels above 1000 ng/ml have recently been proposed as exclusion criteria in the United States (13), application

Table 4. Comparative analysis according to aetiology of liver disease

Variable	HBV n = 94 (28.7%)	Non-HBV n = 233 (71.3%)	P
Age, years (±SD)	55 ± 8	58 ± 8	0.001
Waiting list, months, median (IQR)	4.0 (1.0–10.0)	3.0 (0.0–7.2)	
MELD, (±SD)	15 ± 6	17 ± 8	0.01
Pretransplant images			
Within Milan, *n* (%)	82 (87.2)	187 (80.3)	0.08
French AFP ≤2/>2, *n* (%)	72 (77.2)/22 (22.8)	187 (80.3)/46 (19.7)	0.31
AFP, ng/ml, median (IQR)			
AFP ≤100 ng/ml, *n* (%)	58 (63.0)	186 (80.5)	0.004
AFP 100–1000 ng/ml, *n* (%)	26 (28.3)	35 (15.2)	0.06
AFP >1000 ng/ml, *n* (%)	8 (8.7)	10 (4.3)	0.001
Treatment during waiting list, *n* (%)	36 (38.3)	119 (51.1)	0.04
Explanted liver features			
Within Milan, *n* (%)	53 (56.4)	149 (63.9)	0.12
Within up to 7, *n* (%)	74 (78.7)	192 (82.4)	0.26
Macrovascular invasion, *n* (%)	5 (6.0)	5 (2.7)	0.16
Microvascular invasion, *n* (%)	28 (30.1)	49 (21.2)	0.11
Nuclear grade >II, *n* (%)	33 (36.7)	42 (22.8)	0.01

Normal values: alpha-fetoprotein 0.6–4.4 ng/ml.
AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

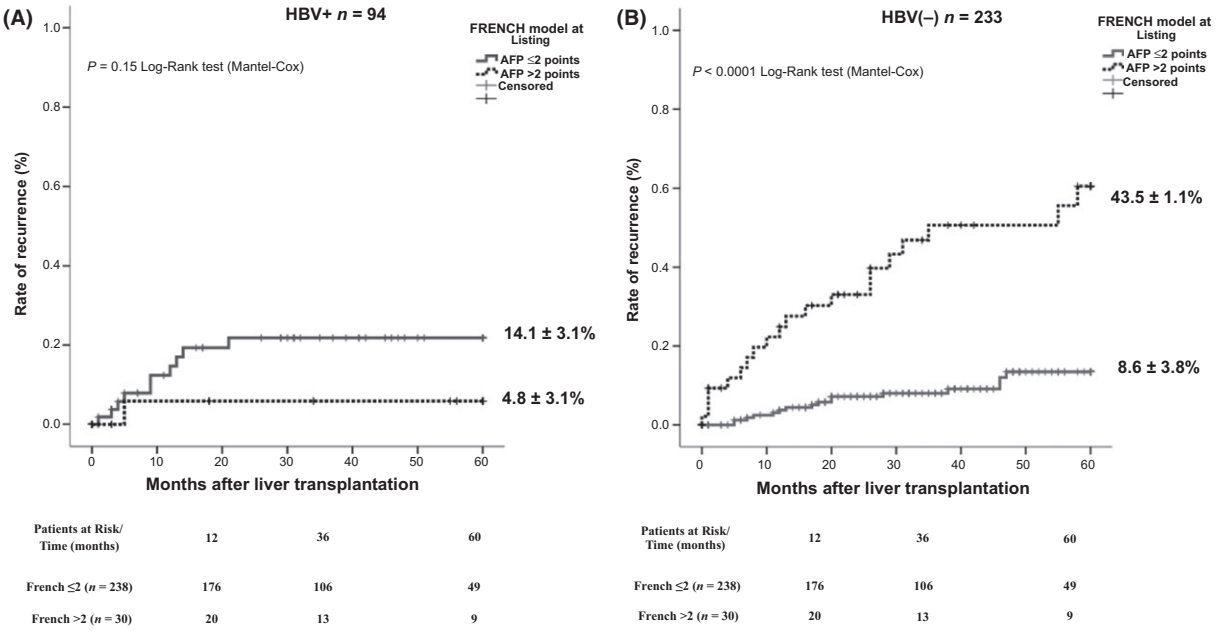

Fig. 4. Tumour recurrence rates according to alpha-fetoprotein (AFP) model in patients with and without chronic hepatitis B infection (Panels A–B).

of this marker to organ allocation policies in Latin America has not been systematically implemented.

Together with what Duvoux *et al.* (14) has previously published, when tested according to the course of risk stratification during the waiting list, those patients moving from AFP >2 points to the low-risk group (≤2 points) after tumour treatment, had similar recurrence risk when compared to patients originally classified in the low-risk group. Our group could not perform this analysis because there were a limited number of patients to be considered for this subgroup comparison.

We acknowledge that specific, population-related differences exist between European and Latin-American cohorts. Firstly, the most common cause of liver disease in Latin America was chronic HBV infection, followed by HCV, whereas in French cohorts, alcoholic cirrhosis followed by HCV were the most frequent causes of

HCC. Indeed, in the Italian cohort, the AFP model performed better for HCV than for HBV patients. In our cohort, although HBV patients presented higher AFP levels, this tumour marker showed significant correlation with recurrence in non-HBV patients, indicating that the AFP model performed better in this subgroup of patients. These results suggest that application of scores such as this one may be population specific and require local validation and calibration.

This study has limitations worthy of mention. Data collection was retrospective; however, all co-authors were blinded to the AFP score results to avoid differential outcome assessment on exposure and a complete follow-up and outcome assessment was available for all patients included, with central quality control of reported data. Secondly, imaging re-evaluation after local/regional treatment was not centrally reviewed, or was a specific method applied (e.g. RECIST, EASL criteria) (29); reason for which is we only evaluated AFP model and MC results for the overall cohort at time of listing, and additionally at most recent evaluation in treated patients. Thirdly, an overlap seems to exist between MC and the AFP model when including assessment of imaging data only. However, as previously shown, one-third of the patients exceeding MC would have been 'rescued' showing good outcomes after LT if the AFP model was considered.

In conclusion, the results observed in this Latin-American cohort were associated with better survival and lower recurrence rates in patients exceeding MC, with AFP scores $\leq$2 points, highlighting the ability of the AFP model to identify patients beyond MC criteria who may nevertheless present excellent outcomes. Although overlapping between MC and the AFP model may occur, the latter could be useful in Latin-American countries, to better select patients for LT in subgroups exceeding MC. However, special attention should be the focus on patients with HBV, as the AFP model may not discriminate degrees of patient risk well enough in this particular population.

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