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Combinations of Biomarkers and Milan Criteria for Predicting Hepatocellular Carcinoma Recurrence After Liver Transplantation

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Abstract

Growing evidence suggests that pretransplant alpha-fetoprotein (AFP) predicts outcomes of hepatocellular carcinoma (HCC) patients treated with liver transplantation. We aimed to determine whether pretransplant AFP, *Lens culinaris* agglutinin-reactive alpha-fetoprotein (AFP-L3), and des-gamma-carboxyprothrombin (DCP) predicted HCC recurrence after transplantation. A retrospective cohort study of 313 HCC patients undergoing transplantation between 2000 and 2008 was conducted, and 48 (15.3%) developed recurrence during a median follow-up of 78.9

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months. The 127 patients with available serum drawn before transplantation were included; they included 86 without recurrence and 41 with recurrence. Serum was tested for AFP, AFP-L3%, and DCP in a blinded fashion with the μ TASWako i30 immunoanalyzer. All biomarkers were significantly associated with HCC recurrence. The hazard ratios (HRs) were 3.5 [95% confidence interval (CI), 1.9–6.7; $P < 0.001$] for DCP ≥ 7.5 ng/mL and 2.8 (95% CI, 1.4–5.4; $P = 0.002$) for AFP ≥ 250 ng/mL. The HR increased to 5.2 (95% CI, 2.3–12.0; $P < 0.001$) when AFP ≥ 250 ng/mL and DCP ≥ 7.5 ng/mL were considered together. When they were combined with the Milan criteria, the HR increased from 2.6 (95% CI, 1.4–4.7; $P = 0.003$) for outside the Milan criteria to 8.6 (95% CI, 3.0–24.6; $P < 0.001$) for outside the Milan criteria and AFP ≥ 250 ng/mL and to 7.2 (95% CI, 2.8–18.1; $P < 0.001$) for outside the Milan criteria and DCP ≥ 7.5 ng/mL. Our findings suggest that biomarkers are useful for predicting the risk of HCC recurrence after transplantation. Using both biomarkers and the Milan criteria may be better than using the Milan criteria alone in optimizing the decision of liver transplantation eligibility.

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second most common cause of cancer deaths worldwide.¹ Treatment with liver transplantation yields an excellent outcome, with a 5-year survival rate of more than 70% in select patients.² Nonetheless, the selection of optimal candidates for transplantation remains practically challenging. Up to 20% of HCC patients develop disease recurrence after transplantation and consequently have poor survival.³ To maximize the efficacy of treatment with transplantation given the current organ shortage, preoperative factors that can reliably predict the risk for recurrence after transplantation are needed.

The Milan criteria are an established predictor of HCC recurrence after transplantation.⁴ Patients whose tumor burden is within the Milan criteria had a low recurrence rate 4 years after transplantation of only 8%, whereas it was 41% for those with a tumor burden outside the Milan criteria.⁴ Despite being a good predictor, the Milan criteria have some limitations. The Milan criteria rely solely on findings from radiologic imaging; thus, their predictive performance can vary with the accuracy of the radiologic tests. Additionally, the Milan criteria do not account for tumor biology, an important factor determining the risk of recurrence after transplantation.⁵ To overcome these limitations, other objective pretransplant parameters that can predict recurrence are required.

A growing body of evidence suggests that alpha-fetoprotein (AFP), the most widely used biomarker for HCC surveillance and diagnosis, also has prognostic utility in HCC patients treated with transplantation.^{3,5–10} It has been consistently shown that elevated pre-transplant serum AFP levels ranging from 200 to 1000 ng/mL are associated with an increased risk of recurrence after transplantation.⁵ This finding has led to a statement in the recent international consensus recommendation for HCC treatment with transplantation that AFP may help in making decisions on eligibility for transplantation when it is used in combination with imaging criteria.⁵ However, a recommended AFP cutoff for this decision has not been established yet. The percentage of *Lens culinaris* agglutinin-reactive alpha-fetoprotein (AFP-L3) and des-gamma-carboxyprothrombin (DCP) are additional biomarkers commonly used in conjunction with AFP as HCC surveillance or diagnostic tools in Asia.¹¹ Whether AFP-L3% and DCP can be used as single biomarkers or in combination with AFP

for the prediction of HCC recurrence after transplantation is not well investigated. A few studies, all conducted in Japan, showed that a high DCP level before transplantation was associated with HCC recurrence after transplantation.^{8,9,12,13} This finding has never been validated in a Western population. Moreover, it is not yet known if AFP-L3% can predict HCC recurrence after transplantation.

The primary aim of this study was to determine the association between pretransplant serum DCP and AFP-L3% and the risk of HCC recurrence after transplantation in the US population. The secondary aim was to explore whether a combination of biomarkers is more useful than a single biomarker in predicting the risk of HCC recurrence. Lastly, we aimed to investigate whether the addition of biomarkers improves the performance of the Milan criteria in predicting HCC recurrence after transplantation.

PATIENTS AND METHODS

Study Population

The study was approved by the Mayo Clinic institutional review board. Patients with HCC who underwent liver transplantation at 2 Mayo Clinic sites (Rochester, MN, and Jacksonville, FL) between January 2000 and December 2008 were identified (n = 313). The diagnosis of HCC was made with the following criteria: (1) pathology from the explanted liver or (2) a new mass > 1 cm in size developing in a cirrhotic liver and detected by 2 imaging modalities with arterial enhancement and portal venous washout on computed tomography (CT), magnetic resonance imaging, or angiography. Of the 313 HCC patients, 48 (15.3%) had HCC recurrence during a median follow-up of 78.9 months; 127 of the HCC patients, including 41 patients with recurrence and 86 patients without recurrence, had stored serum samples available that were obtained immediately before transplantation. The relative paucity of stored samples for patients without recurrence was due to preferential use of nonrecurrent patient samples in prior studies using the Liver Transplant Biobank, and this led to a depletion of a larger proportion of nonrecurrent samples versus recurrent patient samples. To ensure that this depletion did not result in a bias in this study, we examined the demographic and tumor characteristics of the nonrecurrent patients in the cohort with available serum samples and those without serum samples. There was no significant difference between the 2 cohorts (Supporting Table 1).

The demographics, etiology of liver disease, date of HCC diagnosis, baseline tumor characteristics at the time of the HCC diagnosis [maximum tumor size, tumor number, and vascular invasion (defined as tumor soft tissue invading and partially or completely occluding a vessel)] assessed by cross-sectional CT and/or MRI imaging, liver and renal function tests, Model for End-Stage Liver Disease (MELD) score, bridging locoregional therapy (transarterial chemoembolization, radiofrequency ablation, or others) before transplantation, response to bridging therapy, pathological report for the explant liver, recurrence, and last follow-up visit were retrospectively abstracted from the electronic medical record. The Milan criteria were determined by pretransplant radiologic imaging at the time of the HCC diagnosis. Patients were considered to be within the Milan criteria if they had 1 tumor ≤ 5 cm or 2 to 3 tumors with the size of the largest tumor ≤ 3 cm, no vascular invasion, and no extrahepatic metastasis. A response to bridging therapy was

defined according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST; ie, the relative arterial enhancement and/or summation of the unidimensional diameter of tumors after treatment was determined).¹⁴ The available CT and/or MRI imaging was reviewed by a hepatobiliary radiologist with experience in determining the mRECIST response to locoregional therapy. For patients who had more than 1 session of locoregional therapy before transplantation, the last imaging study before transplantation was assessed for the mRECIST response (ie, interval imaging between the first locoregional therapy and transplantation was ignored).¹⁵ Patients who had a complete or partial response by mRECIST were classified as responders, and those who had stable disease or progressive disease by mRECIST were classified as nonresponders.¹⁵

Measurement of Biomarkers

Serum samples were stored at -80°C until testing for AFP, AFP-L3, and DCP. The assays were performed according to the manufacturer's protocols by an immunoassay technologist using the $\mu\text{TASWako i30}$ instrument (Wako Life Sciences Inc., Mountain View, CA). The $\mu\text{TASWako i30}$ uses a liquid-phase binding assay for measuring AFP, AFP-L3, and DCP simultaneously. In the previous Wako LiBASys instrument, AFP-L3% could not be reported if the total AFP level was < 10 ng/mL. With the new $\mu\text{TASWako i30}$ instrument, AFP-L3% can be reported if the total AFP level is > 0.6 ng/mL. AFP-L3 was reported as a percentage of the total AFP by the machine. The absolute AFP-L3 level was computed as $\text{AFP-L3\%} \times \text{total AFP value}$. The reportable ranges for AFP, AFP-L3, and DCP on the $\mu\text{TASWako i30}$ were 0.3 to 1000 ng/mL, 0.5% to 99.5%, and 0.1 to 950 ng/mL, respectively. If bio-marker levels were above the upper limit of detection, serial dilution was performed to determine the actual level of biomarkers in the serum samples. The inter-assay coefficient of variation was 0.7% to 1.5% for AFP, 0.3% to 5.6% for AFP-L3%, and 1.3% to 7.9% for DCP.

Statistical Analysis

Data were analyzed with SAS 9.1 (SAS Institute, Cary, NC). The 127 patients who had serum samples available were included in the analysis. Biomarker levels were reported as medians and ranges. The percentile of the ranked biomarker values was plotted against the hazard ratio (HR) of recurrence with a smoothing spline function, and the observed pattern was used to categorize the biomarker values for further analysis.

Biomarkers and variables potentially associated with HCC recurrence after liver transplantation were identified with univariate Cox proportional hazards regression analysis. Variables with $P < 0.05$ in the univariate models were included in the multivariate model. The HR and corresponding 95% confidence interval (CI) with concordance scores were reported, and $P < 0.05$ was considered significant.

RESULTS

Baseline Characteristics and Serum Biomarker Levels

Table 1 displays the baseline characteristics of the 127 HCC patients with available serum samples. The median levels of each biomarker for those with recurrence ($n = 41$) and those without recurrence ($n = 86$) were 20.1 ng/mL (range, 0.9–27,800 ng/mL) and 22.9 ng/mL

(range, 0.8–2,020 ng/mL) for AFP ($P = 0.36$), 21.6% (range, 1.0%–88.0%) and 10.8% (range, 1.1%–86.5%) for AFP-L3% ($P = 0.01$), 6.4 ng/mL (range, 0.009–22,518 ng/mL) and 2.0 ng/mL (range, 0.008–850 ng/mL) for absolute AFP-L3 ($P = 0.11$), and 1.7 ng/mL (range, 0.2–1,480 ng/mL) and 0.7 ng/mL (range, 0.2–217 ng/mL) for DCP ($P = 0.005$).

To better understand the impact of biomarker values on the risk of HCC recurrence, we assessed the overall shape of the relationship between biomarker values and the risk of HCC recurrence with a smoothing spline function. On the smoothing spline plots, there was little association between the levels of all bio-markers and the risk of HCC recurrence up till approximately the 80th percentile of each biomarker. The risk of recurrence then substantially increased with bio-marker levels above the 80th percentile for all bio-markers (Supporting Figs. 1–4). The 80th percentile values were 230 ng/mL for AFP, 37% for AFP-L3%, 56 ng/mL for absolute AFP-L3, and 7.5 ng/mL for DCP. On the basis of these findings, all the biomarkers evaluated were categorized into 2 groups with the 80th percentile value as a cutoff for further analysis.

Associations Between Baseline Variables and Risk of HCC Recurrence After Liver Transplantation

We examined the association between baseline patient and pretransplant tumor characteristics and HCC recurrence. In the univariate Cox proportional hazards model, the tumor size and the Milan criteria were significantly associated with HCC recurrence. HRs (95% CI) were 1.4 (95% CI, 1.1–1.7; $P = 0.004$) for tumor size and 2.6 (95% CI, 1.4–4.7; $P = 0.003$) for the Milan criteria. Other variables, namely, age, sex, etiology of liver chronic liver disease, tumor number, vascular invasion, MELD score, and liver and renal function test values, were not associated with an increased risk of recurrence (Supporting Table 2).

Association Between Biomarker Levels and Risk of HCC Recurrence After Liver Transplantation

Next, we determined the association between bio-marker levels and the risk of HCC recurrence. With the 80th percentile value as a cutoff, all biomarkers were significantly associated with the risk of HCC recurrence with HRs ranging from 2.4 to 3.5. DCP 7.5 ng/mL had the largest HR of 3.5 (95% CI, 1.9–6.7; $P < 0.001$), and this suggested that DCP was the most powerful predictor of HCC recurrence among the 4 biomarkers evaluated. The HRs (95% CI) were 3.2 (95% CI, 1.7–6.1; $P < 0.001$) for AFP-L3 37%, 2.6 (95% CI, 1.3–5.0; $P = 0.005$) for AFP 230 ng/mL, and 2.4 (95% CI, 1.2–4.7; $P = 0.01$) for absolute AFP-L3 56 ng/mL.

For the purposes of clinical use and future validation studies, more convenient cutoffs for AFP, AFP-L3%, and absolute AFP-L3 were established: 250 ng/mL, 35%, and 50 ng/mL, respectively. The convenience cutoff for DCP remained at the 80th percentile value of 7.5 ng/mL. The HRs of these convenience cutoffs are shown in Table 2. After adjustments for the Milan criteria, the HRs of all biomarkers remained significant with very little change in the magnitude of the HRs (data not shown). The model did not adjust for tumor size because the tumor size variable is a component of the Milan criteria.

We also explored whether the risk of recurrence increased further if the biomarker levels were above the 90th percentile. The HRs of biomarker levels between the 80th and 90th percentiles and those of levels greater than the 90th percentile were estimated with the biomarker level of < 80th percentile as a reference group. Interestingly, the risk of HCC recurrence was greater in those with biomarker levels above the 90th percentile than those with biomarker levels between the 80th and 90th percentiles for AFP-L3%, absolute AFP-L3, and DCP, but not for AFP. When this was compared to AFP-L3% values < 37%, the HR (95% CI) increased from 2.6 (95% CI, 1.2–6.6; $P = 0.01$) for AFP-L3% values of 37% to 52% to 3.7 (95% CI, 1.7–8.1; $P = 0.001$) for AFP-L3% values > 52%. Similarly, when this was compared to absolute AFP-L3 values < 56 ng/mL, the HR (95% CI) rose from 2.2 (95% CI, 0.8–5.6; $P = 0.1$) for absolute AFP-L3 values of 56 to 300 ng/mL to 2.5 (95% CI, 1.1–5.8; $P = 0.03$) for absolute AFP-L3 values > 300 ng/mL. Notably, when this was compared to DCP values < 7.5 ng/mL, the HR (95% CI) of the DCP increased markedly from 2.6 (95% CI, 1.1–6.0; $P = 0.03$) for DCP values of 7.5 to 18 ng/mL to 5.0 (95% CI, 2.3–10.8; $P = < 0.001$) for DCP values > 18 ng/mL.

Finally, the prognostic value of combinations of the biomarkers was evaluated. Table 3 shows the association between AFP and DCP when they were considered together and the risk of HCC recurrence. The risk was additively increased in patients with both AFP ≥ 250 ng/mL and DCP ≥ 7.5 ng/mL in comparison with those having an elevated level of either biomarker alone. The HR (95% CI) was 5.2 (95% CI, 2.3–12.0; $P < 0.001$) for elevations of both AFP and DCP, 2.3 (95% CI, 0.9–6.1; $P = 0.1$) for an elevation of AFP alone, and 3.2 (95% CI, 1.4–7.4; $P = 0.005$) for an elevation of DCP alone. These HRs remained significant with slight changes in magnitude after adjustments for the Milan criteria (data not shown). This suggests that a combination of biomarkers may be more useful than a single bio-marker for predicting the risk of HCC recurrence.

Association Between Combined Biomarker and Milan Criteria and Risk of HCC Recurrence

To determine whether combinations of each bio-marker and the Milan criteria were associated with HCC recurrence, patients were categorized into 4 groups on the basis of the biomarker value at the designated cutoff and the Milan criteria. Not surprisingly, patients with a tumor size outside the Milan criteria and with elevated biomarker levels had high risks for recurrence, with HRs ranging between 3.6 and 8.6 (Fig. 1). For patients with tumors outside the Milan criteria, an AFP level ≥ 250 ng/mL conferred the highest risk of recurrence among the 4 biomarkers evaluated (HR, 8.6; 95% CI, 3.0–24.6; $P < 0.001$).

Notably, for patients who had tumors within the Milan criteria, those with biomarker levels above the cutoff had a significantly greater risk of recurrence than those with biomarker levels below the cutoff. Among patients with tumors within the Milan criteria, the HRs (95% CI) were 3.2 (95% CI, 1.3–7.5; $P = 0.01$) for AFP ≥ 250 ng/mL, 4.3 (95% CI, 1.8–10.1; $P < 0.001$) for DCP ≥ 7.5 ng/mL, 4.5 (95% CI, 1.9–10.6; $P < 0.001$) for AFP-L3% ≥ 35, and 4.1 (95% CI, 1.8–9.7; $P = 0.001$) for absolute AFP-L3 ≥ 56. These findings indicate that biomarker values add predictive information to the Milan criteria.

To examine whether combinations of biomarker and Milan criteria were more predictive than the Milan criteria, the concordance indices of the Milan criteria alone and the Milan

criteria in combination with the cutoff value of each biomarker were estimated. We found that the concordance index of the Milan criteria increased when they were combined with any of the 4 biomarkers, and this suggests that combining the bio-markers with the Milan criteria was more predictive of recurrence than the Milan criteria alone. The concordance indices were 0.63 (95% CI, 0.56–0.70) for the Milan criteria, 0.68 (95% CI, 0.60–0.76) for the Milan criteria with AFP, 0.70 (95% CI, 0.62–0.78) for the Milan criteria with DCP, 0.70 (95% CI, 0.62–0.78) for the Milan criteria with AFP-L3%, and 0.68 (95% CI, 0.60–0.76) for the Milan criteria with absolute AFP-L3.

DISCUSSION

In this study, we found significant associations between pretransplant serum AFP, AFP-L3%, absolute AFP-L3, and DCP levels and the risk of HCC recurrence after transplantation. DCP was the strongest predictor for disease recurrence among the 4 biomarkers evaluated, and it was also a stronger predictor than the Milan criteria. The recurrence risk was additively increased when AFP and DCP were considered together, and this supports the concept that there is no perfect single biomarker because HCC is a highly heterogeneous tumor; thus, the combination of biomarkers is more informative than any single bio-marker alone.¹¹ More importantly, when used in combination with the Milan criteria, all biomarkers improved the performance of the Milan criteria in the prediction of risk of recurrence after transplantation.

A recent systematic review has shown that pretransplant AFP is associated with the risk of HCC recurrence after transplantation, with varied risk magnitudes depending on the defined cutoffs.⁶ In the present study, an AFP cutoff of 250 ng/mL was associated with a 2.8-fold increased risk of HCC recurrence after transplantation. A recent report showed that a maximum value of pretransplant AFP at a cutoff of 253 ng/mL had a good performance in predicting HCC recurrence after living donor liver transplantation; it yielded an area under the receiver operating characteristic curve of 0.878, a sensitivity of 73%, and a specificity of 91%.⁹ Additionally, a pre-transplant AFP level > 200 ng/mL was found to be significantly correlated with a higher incidence of microvascular invasion in the explant liver.⁸ All these findings suggest that AFP at the cutoff of 250 ng/mL could potentially be a good preoperative predictor of HCC recurrence after transplantation. Additional studies to confirm the generalizability of this cutoff are warranted.

In agreement with previous reports, we found that DCP was a stronger predictor of recurrence than AFP.^{8,9,12,13} This is biologically plausible because DCP binds to vascular endothelial growth factor receptor 2 and induces autophosphorylation of the receptor and downstream effectors, including phospholipase C- γ and mitogen-activated protein kinase; this consequently promotes endothelial cell proliferation and migration.¹⁶ However, AFP is a product of tumor cells rather than having any biological effect to promote tumor growth. Similar to DCP, AFP-L3% was shown to be associated with portal vein invasion in a prospective study from the United States.¹⁷ We found that both AFP-L3% and absolute AFP-L3 were significantly associated with HCC recurrence after transplantation, with a magnitude of risk comparable to that of AFP and DCP. This finding needs further validation in another cohort.

As expected, the Milan criteria were a significant predictor of HCC recurrence after transplantation. The recurrence risk increased markedly if patients had both tumors outside the Milan criteria and a high level of any of the biomarkers. More importantly, patients who had tumors within the Milan criteria with elevated biomarker levels also had a significantly increased risk for recurrence, with HRs ranging from 3.2 to 4.5 that were even higher than the HR of 2.6 for being outside the Milan criteria. Our findings were in line with a recent report showing that a model including both the Milan criteria and the pretransplant AFP value better predicted the recurrence risk than the Milan criteria alone.¹⁰ Thus, our study along with the previous report strongly suggests that bio-marker values add predictive information to the Milan criteria and can be used in a complementary fashion with the Milan criteria to select optimal candidates for liver transplantation.

It is notable that the proportion of blood samples available from patients with recurrence after liver transplantation [41 of 127 (32%)] was substantially higher than the proportion of patients with recurrence in the entire cohort of HCC patients receiving liver transplants [48 of 313 (15%)]. The percentage of patients with recurrence in the entire transplanted HCC cohort was comparable to those reported at other referral centers.^{15,18} We investigated the possible causes for the low availability of serum samples from nonrecurrent HCC patients in our cohort. It appears that the discrepancy was likely due to the depletion of samples used in studies conducted before our study. Consultation with the director of the transplant biorepository at the Mayo Clinic revealed that there have been more requests for serum from recipients without HCC recurrence over the past several years. We suspect that this has led to a systematic bias; that is, these samples were partially depleted by studies pulling serum samples before our study. However, because of the retrospective nature of the study design, a selection bias could not be totally excluded. To account for this possible bias, an analysis adjusting for the etiology of liver disease was performed. The results were consistent with those of the original analysis (data not shown). This discrepancy could raise a concern about whether the biomarker performance results obtained from the 127 patients can be extrapolated to the entire cohort of HCC patients undergoing liver transplantation. To answer this question, we compared the baseline patient and tumor characteristics of the 127 patients who had serum samples available versus the 186 patients who did not have serum samples available for biomarker measurement (Supporting Table 3). Both groups had similar baseline patient and tumor characteristics for all variables, and this suggested that the results observed in the 127 patients with samples were likely to be applicable to the 186 patients for whom serum samples were not available.

There are a number of strengths of this study. First, we have described the novel finding that biomarkers alone or in combination with the Milan criteria were strong predictors of HCC recurrence after transplantation. Because biomarker measurement is a simple test that can easily be done before transplantation, our findings could, therefore, potentially improve the organ allocation algorithm for liver transplantation. It will be important to further investigate whether incorporating multiple biomarkers with or without other objective preoperative parameters into the Milan criteria would provide better predictive performance than combining a single biomarker with the Milan criteria. Second, because of very limited prior evidence of the utility of AFP-L3% and DCP for predicting the risk of HCC recurrence after transplantation, particularly in Western populations, our study fills an important knowledge

gap in this regard. Lastly, the biomarker levels were measured with the new instrument μ TASWako i30, which provides substantial improvements in sensitivity over previous instruments. This will potentially be particularly useful in the transplant setting because these patients generally have early-stage disease, in which tumor biomarker levels are often not highly elevated.

There are a number of limitations to our study. Because of the relatively small sample size, we were not able to adjust for all tumor- and treatment-related factors that may have influenced recurrence in the analysis. We, therefore, chose to examine only the Milan criteria in addition to the biomarkers because the Milan criteria represent a composite of the critical tumor-related factors determining the risk of recurrence. The mRECIST response to transarterial chemoembolization was recently shown to be significantly related to HCC recurrence after liver transplantation.¹⁵ Despite having only a limited amount of data because of the overall small sample size, we were able to show that after adjustments for the mRECIST response, the HRs for the risk of recurrence were of similar magnitudes to the unadjusted HRs (see the supporting data). Also, the correlation of tumor histological characteristics and pathologic Milan criteria and biomarker levels was not included in the main analysis because our focus was to identify preoperative predictors of recurrence. Nonetheless, after adjustments for the pathological Milan criteria, the biomarker levels remained significantly associated with a high recurrence risk, with HRs comparable to those observed in the main analysis (see the supporting data). It was recently shown that a rise in the natural AFP slope before transplantation predicted HCC recurrence after transplantation.⁷ Unfortunately, we were not able to validate the predictive performance of a trend in biomarker value changes in our cohort because only a few patients had available blood samples drawn multiple times before transplantation. It will be important to determine whether our findings can be generalized to other patient populations. If this result can be replicated in another larger cohort, it may improve decision making for organ allocation for liver transplantation. Establishing the optimal cutoffs of the biomarker(s) that will have the most predictive ability across different cohorts of HCC patients eligible for transplantation is the next step before the use of these biomarkers can be implemented in practice.

In conclusion, incorporating pretransplant AFP, AFP-L3, and DCP levels into the Milan criteria improves the performance of the Milan criteria in predicting HCC recurrence after transplantation. This could potentially help define a better strategy for organ allocation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AFP	alpha-fetoprotein
AFP-L3	<i>Lens culinaris</i> agglutinin-reactive alpha-fetoprotein
CI	confidence interval
CT	computed tomography
DCP	des-gamma-carboxyprothrombin
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HR	hazard ratio
INR	international normalized ratio
MELD	Model for End-Stage Liver Disease
mRECIST	modified Response Evaluation Criteria in Solid Tumors
PT	prothrombin time
SD	standard deviation

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Figure 1.
Association between combinations of biomarkers and the Milan criteria and the risk for HCC.

TABLE 1

Baseline Characteristics of 127 HCC Patients Undergoing Liver Transplantation for Whom Pretransplant Serum Samples Were Available

Variables	Value
Age, years, mean $\pm$ SD	58.4 $\pm$ 8.2
Male, n (%)	99 (78.0%)
Race, n (%)	
White	99 (78.0%)
Nonwhite	25 (19.7%)
Unknown	3 (2.4%)
Etiology, n (%)	
HCV	76 (59.8%)
HBV	11 (8.7%)
Nonalcoholic fatty liver disease	5 (3.9%)
Alcohol	17 (13.4%)
Other	18 (14.2%)
MELD score, mean $\pm$ SD	12.5 $\pm$ 9.0
Log _e bilirubin, mg/dL, mean $\pm$ SD	0.6 $\pm$ 0.8
Albumin, g/dL, mean $\pm$ SD	3.3 $\pm$ 0.6
INR, mean $\pm$ SD	1.3 $\pm$ 0.2
PT, seconds, mean $\pm$ SD	15.0 $\pm$ 2.9
Creatinine, mg/dL, mean $\pm$ SD	0.9 $\pm$ 0.3
AFP, ng/mL, median (range)	22.1 (0.8–27,800)
AFP $\geq$ 250 ng/mL, n (%)	25 (19.7%)
AFP-L3%, median (range)	12.4 (1–88)
AFP-L3% $\geq$ 35%, n (%)	26 (20.5%)
Absolute AFP-L3, ng/mL, median (range)	3.6 (0.008–22,518)
Absolute AFP-L3 $\geq$ 50 ng/mL, n (%)	26 (20.5%)
DCP, ng/mL, median (range)	0.8 (0.2–1,480)
DCP $\geq$ 7.5 ng/mL, n (%)	27 (21.3%)
Maximum tumor size, cm, mean $\pm$ SD	3.1 $\pm$ 1.4
Tumor size $>$ 3 cm, n (%)	57 (44.9%)
Tumor number, mean $\pm$ SD	1.8 $\pm$ 0.9
Multiple tumor lesions, n (%)	65 (51.2%)
Presence of vascular invasion, n (%)	3 (2.4%)
Milan criteria, n (%)	
Within Milan criteria	86 (67.7%)
Outside Milan criteria	41 (32.3%)
Waiting time, months, median (range)	2.7 (0.03–20.6)

TABLE 2

Univariate Cox Proportional Hazards Analysis of the Effects of the Milan Criteria and Biomarker Levels on Risk of HCC Recurrence After Liver Transplantation

Variable	HR (95% CI)	P Value
Outside Milan criteria	2.6 (1.4–4.7)	0.003
AFP ≥ 250 ng/mL	2.8 (1.4–5.4)	0.002
AFP-L3% ≥ 35%	3.2 (1.7–6.1)	<0.001
Absolute AFP-L3 ≥ 50 ng/mL	2.4 (1.2–4.7)	0.01
DCP ≥ 7.5 ng/mL	3.5 (1.9–6.7)	<0.001

TABLE 3

Multivariate Cox Proportional Hazards Analysis of the Effects of Combinations of AFP and DCP on Risk of HCC Recurrence After Liver Transplantation

Combinations of Biomarkers				HR (95% CI)	P Value
AFP	250 ng/mL and DCP	7.5 mg/mL		5.2 (2.3–12.0)	< 0.001
AFP < 250 ng/mL and DCP	7.5 mg/mL			3.2 (1.4–7.4)	0.005
AFP	250 mg/mL and DCP < 7.5 mg/mL			2.3 (0.9–6.1)	0.10
AFP < 250 mg/mL and DCP < 7.5 mg/mL				1.0 (reference)	