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ORIGINAL ARTICLE

Evaluation methods for pretransplant oncologic markers and their prognostic impacts in patient undergoing living donor liver transplantation for hepatocellular carcinoma

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Summary

Tumor markers [alpha-fetoprotein (AFP) or des-gamma-carboxyprothrombin (DCP)] and neutrophil/lymphocyte ratio (NLR) reportedly correlate with long-term outcomes for hepatocellular carcinoma (HCC). However, no standardized method has been established for evaluating the pretransplant data. One hundred and twenty-four patients who underwent living donor liver transplantation (LDLT) were retrospectively reviewed. The best predictive parameters for tumor recurrence were maximum values for AFP or DCP and 90-day mean values for NLR, respectively, and multivariate analysis confirmed these values were correlated with tumor recurrence. However, receiver operating characteristic analysis revealed that discriminative powers were sufficient only in maximum AFP [area under the curve (AUC) 0.88, $P < 0.001$] and maximum DCP (AUC 0.76, $P < 0.001$), while mean NLR was less predictive (AUC 0.62, $P = 0.20$). When incorporating AFP and DCP to the Tokyo criteria (≤5 tumors with each tumor ≤5 cm), the presence of at least two of the following factors: (i) beyond the Tokyo criteria, (ii) AFP>250 ng/ml, and (iii) DCP > 450 mAu/ml (>450 ng/ml), was correlated with a worse 5-year disease-free survival rate (20.0% vs. 96.8%, $P < 0.001$) and 5-year overall survival rate (20.0% vs. 84.0%, $P < 0.001$). The prognosis of patients undergoing LDLT for HCC strongly relies on maximum AFP or DCP values before transplantation, while the prognostic impact of NLR is limited.

Introduction

Serum levels of tumor markers [1–5] and inflammatory parameters [6–9] are reported to correlate with survival outcomes of hepatocellular carcinoma (HCC) in patients undergoing liver resection and liver transplantation (LT). In the clinical setting, however, it is difficult to define the pretransplant oncologic status of patients with only a one-point evaluation of tumor markers because serum levels of alpha-fetoprotein (AFP) and des-gamma-carboxyprothrombin (DCP) are highly influenced by pretransplant treatments for HCC, medications such as vitamin K or Warfarin, and the fibrotic status of the underlying liver. Also, the inflammatory status, represented by the neutrophil/lymphocyte ratio (NLR), is often unstable due to the compromised condition of the recipients with end-stage liver disease, and accordingly, its prognostic value remains controversial [10].

The clinical relevance of these oncologic/inflammatory parameters is conventionally studied based on the one-time measurement just prior to transplantation. Considering the inherent individual variability of the parameters caused by various pretransplant clinical conditions [11–13], there is no solid evidence with regard to (i) whether the one-point measurement of these parameters just prior to transplantation is reliable, (ii) the extent to which these

parameters contribute to survival outcomes, and (iii) the actual predictive power and clinical impact of these parameters. Thus, in this study, we aimed to develop an adequate method of pretransplant evaluation of tumor markers and inflammatory parameters and to test the prognostic values of these parameters for patients undergoing LT for HCC.

Patients and methods

Patients

A total of 500 adult patients underwent living donor liver transplantation (LDLT) at our institution between January 1996 and December 2012. Of these, 124 patients were identified as having HCC during the pretransplant work-up and were studied in detail.

Perioperative case and immunosuppression protocol

Our surgical technique and basic perioperative care are described in detail elsewhere. Living donors were selected after considering their age, blood type, graft size, liver function, and confirming their desire to volunteer [14]. Immunosuppression began immediately after transplantation with tacrolimus (Prograf®, Astellas Pharma Inc., Tokyo, Japan) and steroid in every patient, irrespective of the etiology of end-stage liver disease [15]. If some severe adverse events such as convulsion, encephalopathy, renal insufficiency, or thrombotic microangiopathy were observed, tacrolimus was converted to cyclosporine (Neoral®, Novartis Pharma K.K., Tokyo, Japan). Also, if needed, 2000–3000 mg of mycophenolate mofetil (CellCept®, Chugai Pharmatheutical Co., Ltd., Tokyo, Japan) or basiliximab, an anti-CD25 antibody (Simulect®, Novartis Pharma K.K.), was added with dose reduction or discontinuation of calcineurin inhibitors.

Diagnosis and follow-up of hepatocellular carcinoma

All the patients were referred to our department for the purpose of LDLT mainly due to end-stage liver disease (Child-Pugh C) regardless of the history of HCC. Pretransplant work-up includes screening of viable HCC as well as meticulous assessment of comorbidities. Because donor's safety is paramount in LDLT, we usually take 2–3 months (75 days in median) for pretransplant work-up both for donors and recipients when viable HCC is not confirmed at the time of consultation. However, when emergence of viable HCC was confirmed at the time of consultation or during the work-up duration, LDLT was performed within 1 month after the diagnosis because locoregional treatment is usually not tolerable in these patients with Child-Pugh C hepatic functional reserve.

Final diagnosis and degree of extension of HCC was based on dynamic CT performed within 1 month before LDLT in all cases. Lesions presenting with typical dynamic enhancement pattern (i.e., enhancement in arterial phase and low density during portal phase) were diagnosed as HCC.

Post-transplant follow-up was performed with tumor markers (AFP and DCP) measured every month, ultrasound performed every 2–3 months, and contrast enhanced CT every 4–6 months. Recurrence was defined as emergence of radiological findings in dynamic CT compatible with typical enhancement pattern.

Assessment of tumor markers and inflammatory status

AFP and DCP were measured as a part of the preoperative work-up to confirm the diagnosis of HCC. Pretransplant inflammatory status was also evaluated using the NLR. The trend of the changes in these parameters was reviewed and analyzed beginning from the point of consultation to transplantation.

Data analysis

Statistical analysis was performed using the IBM SPSS software (ver19.0 SPSS Inc, Chicago, IL, USA). Medians and ranges of continuous data were compared using the Mann–Whitney *U*-test. Categorical data were compared using Pearson's chi-squared test or Fisher's exact test, as appropriate. *P*-values of less than 0.05 were considered statistically significant. Survival curves were generated using the Kaplan–Meier method and compared using the log-rank test.

First, to determine the best method of data interpretation for AFP, DCP, and NLR, Wald statistics in a univariate logistic regression analysis for tumor recurrence were compared among (i) pretransplant values on the day before transplantation, (ii) maximum values within 90 days before transplantation, and (iii) mean values within 90 days before transplantation. The parameters with the largest Wald statistics for each respective marker were then used in subsequent prognostic analyses. The risk factors for tumor recurrence were analyzed using a multivariate Cox proportional hazard model with backward elimination, using variables with $P < 0.1$ in the univariate analysis. The optimal cutoff value for each pretransplant oncologic/inflammatory marker was determined using receiver operating characteristic (ROC) analysis, and their ability to stratify long-term outcomes was tested. All the analyses in this study were performed in accordance with the ethical guidelines for clinical studies established at the University of Tokyo Hospital.

Results

Overview

Baseline characteristics of the 124 patients are summarized in Table 1. One hundred and fourteen (92%) patients had viral hepatitis, and 55 (44%) patients had a history of treatment for HCC prior to consultation. Forty-four (35%) patients had HCC beyond the Milan criteria [16], 37 (31%) patients were out of UCSF criteria [17], and 15 (12%) patients exceeded the Tokyo criteria (≤5 tumors, each with a maximum diameter ≤5 cm) [18]. Four patients died within 90 days due to severe complications (heart failure, $n = 2$; sepsis, $n = 1$; and cerebral hemorrhage, $n = 1$), and these patients were excluded from the prognostic analysis. With a median follow-up period of 101.9 months (range, 3.5–165.4 months), 1-year, 3-year, and 5-year overall survival rates of the entire cohort were estimated as 92.4%, 82.5%, and 78.6%, respectively (actual survival rates of 1 year, 92.3%; 3 year, 81.9%; and 5 year, 76.8%, respectively). Recurrence was observed in 11/120 (9%) patients with a median time to recurrence of 8.5 months (range, 3.1–28.2 months). Of the 11 patients, two developed metachronous lung metastases at 23.9 and 28.2 months after transplantation, and both of these patients are currently alive with no evidence of disease for 67.2 and 76.0 months after curative lung resections, respectively. The remaining nine patients had early recurrence within 1 year after transplantation and died at a median of 5.3 months (range, 2.6–32.3 months) after tumor recurrence.

Table 1. Patient characteristics.

N	124
Age	56 (37–67)
Sex (male/female)	98/26
MELD score	12 (2–34)
Etiology	
Hepatitis B (%)	37 (30)
Hepatitis C (%)	75 (60)
Hepatitis B + C (%)	2 (2)
Alcohol (%)	5 (4)
Others (%)	5 (4)
History of treatment prior to consultation	
TACE	49 (40)
RFA	5 (4)
Radiotherapy	1 (1)
Graft type	
Right liver (%)	87 (70)
Left liver (%)	33 (27)
Right lateral sector (%)	4 (3)
Maximum size of tumor, mm	22 (5–300)
Number of lesions	2 (1–16)
Beyond Milan criteria*	44 (35)
Beyond UCSF criteria†	37 (31)
Beyond Tokyo criteria‡	15 (12)
Tumor differentiation	
Well (%)	47 (38)
Moderate (%)	67 (54)
Poor (%)	10 (8)
Microvascular invasion (%)	15 (13)
Pretransplant AFP, ng/ml, median (range)	15 (1–11 999)
% versus normal upper limit, median (range)	150% (10–1110%)
Pretransplant DCP, mAu, ml, median (range)	29 (8–726)
% versus normal upper limit, median (range)	73% (20–18%)
Pretransplant NLR, median (range)	2.4 (0.3–22.3)
Operation time, min	890 (607–1890)
Blood loss, ml	5556 (920–53 835)
Amount of red cell transfusion, ml	2400 (0–14 640)
Length of hospital stay, days	43 (15–176)
90-day mortality (%)	4 (3)

MELD, model for end-stage liver disease; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; AFP, alpha-fetoprotein; DCP, des-γ-carboxyprothrombin; NLR, neutrophil/lymphocyte ratio.
Figures represent median (range) unless indicated.
*Solitary tumor ≤5 cm or ≤3 tumors with each tumor ≤3 cm.
†Solitary tumor ≤6.5 cm or ≤3 tumors with each tumor ≤4.5 cm or total tumor diameter less than 8.5 cm.
‡≤5 tumors with each tumor ≤5 cm.

Pretransplant tumor markers and neutrophil/lymphocyte ratio

Median number of measurements of tumor markers and neutrophil/lymphocyte ratio was 3 (range, 1–9). To determine an adequate method for pretransplant interpretation of oncologic/inflammatory markers, we first compared the discriminatory values of several types of parameters for AFP, DCP, and NLR (Table 2). Univariate regression analysis for tumor recurrence revealed that maximum AFP level, maximum DCP level, and mean NLR were the most predictive parameters for pretransplant oncologic/inflammatory markers. Therefore, these parameters were used in the subsequent prognostic analyses for recurrence.

Table 2. Predictive value of various types of pretransplant oncologic/inflammatory markers for tumor recurrence.

Parameters	Wald χ^2	*P*
AFP		
AFP at -1POD	4.37	0.037
Maximum AFP within 90 days	5.34	0.021
Average AFP within 90 days	3.03	0.818
DCP		
DCP at -1POD	5.69	0.017
Maximum DCP within 90 days	15.01	<0.001
Average DCP within 90 days	8.24	0.004
NLR		
NLR at -1POD	1.88	0.171
Maximum NLR within 90 days	3.01	0.083
Average NLR within 90 days	3.70	0.054

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; NLR, neutrophil/lymphocyte ratio.

Risk factors for tumor recurrence and optimal cutoff value for the oncologic markers

In multivariate analysis, not matching the Tokyo criteria (≤5 tumor or ≤5 cm), the presence of microvascular invasion, maximum AFP level, maximum DCP level, and pretransplant mean NLR were correlated with tumor recurrence (Table 3). Based on the results, the optimal cutoff point and diagnostic values for AFP, DCP, and NLR were determined using ROC analysis (Fig. 1). The best cutoff values for predicting post-transplant recurrence were AFP >253 ng/ml and DCP >449 mAu/ml (>449 ng/ml) with an area under the curve (AUC) of >0.75 and asymptotic significance (compared to AUC = 0.500) of $P < 0.0001$, while the AUC for NLR was relatively small and its discriminatory value was poor ($P = 0.201$; Table 4). When comparing disease-free survival and overall survival using these cutoff points for AFP and DCP, a remarkable prognostic difference was confirmed in both disease-free survival and overall survival (Fig. 2).

Correlation between AFP or DCP levels and histopathologic aggressiveness of HCC

When comparing the histopathologic aggressiveness of HCC based on the cutoff values for AFP and DCP, microvascular invasion was significantly more frequent both in higher AFP (40% vs. 10%, $P = 0.006$) and higher DCP (38% vs. 11%, $P = 0.017$). Also, the incidence of poor tumor differentiation tended to be more frequent with both higher AFP (18% vs. 7%, $P = 0.15$) and higher DCP (23% vs. 7%, $P = 0.08$).

Table 3. Multivariate analysis for pretransplant risk factors for post-transplant recurrence of HCC.

Factors	Univariate *P*-value	Multivariate *P*-value	HR	95% CI
Age >50	0.976			
Sex	0.944			
MELD score >15	0.803			
Viral hepatitis	0.314			
Beyond Tokyo criteria	0.003	0.028	6.44	1.24–34.8
Poor differentiation	<0.001	0.159		
Microvascular invasion	0.003	0.046	7.93	1.04–53.6
Maximum AFP +100 ng/ml	0.014	0.004	1.03	1.01–1.05
Maximum DCP +100 mAu/ml	<0.001	0.001	1.10	1.03–1.22
Mean NLR +1	0.033	0.011	1.26	1.06–1.62
History of treatment for HCC before consultation	0.275			
Pretransplant hemodialysis	0.653			

HCC, hepatocellular carcinoma; HR, hazard ratio; 95% CI, 95% confidence interval; MELD, model for end-stage liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; DCP, des-γ-carboxyprothrombin; NLR, neutrophil/lymphocyte ratio.

New criteria for patients undergoing living donor liver transplantation for HCC

When incorporating pretransplant maximum serum AFP and DCP levels in Tokyo criteria as indicated in Table 5, this scoring system further stratified patients based on the risk of tumor recurrence and poor prognosis. The prognos-

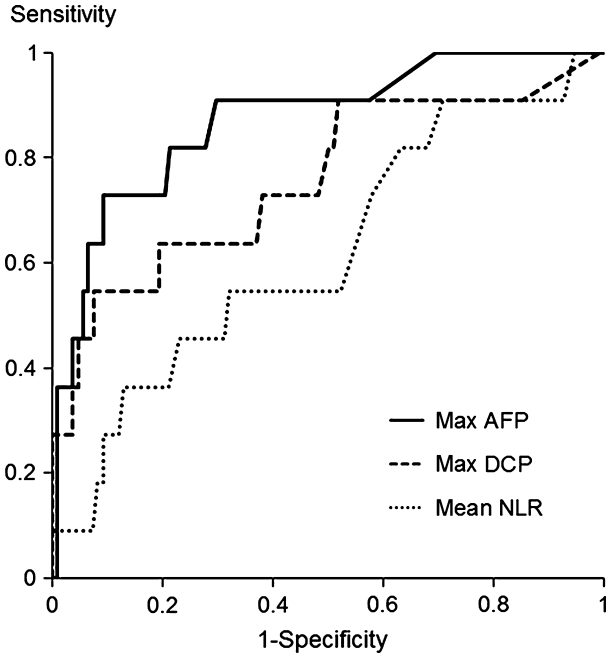

Figure 1 Receiver operating characteristic curves for pretransplant oncologic/inflammatory markers for predicting tumor recurrence. AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin; NLR, neutrophil/lymphocyte ratio.

Table 4. Predictive value of serum markers for post-transplant recurrence of HCC.

	Max AFP	Max DCP	Mean NLR
AUC	0.878	0.756	0.617
SE	0.053	0.090	0.092
Asymptotic significance	<0.0001	<0.0001	0.201
Best cutoff value	253 ng/ml	449 mAu/ml	6.4
Sensitivity	0.727	0.546	0.364
Specificity	0.907	0.926	0.881
Accuracy	0.891	0.891	0.833
LR+	7.82	7.38	3.06
LR-	3.32	2.04	1.39

AUC, area under the curve; SE, standard error; AFP, alpha-fetoprotein; DCP, des-γ-carboxyprothrombin; NLR, neutrophil/lymphocyte ratio; LR+, positive likelihood ratio; LR-, negative likelihood ratio.

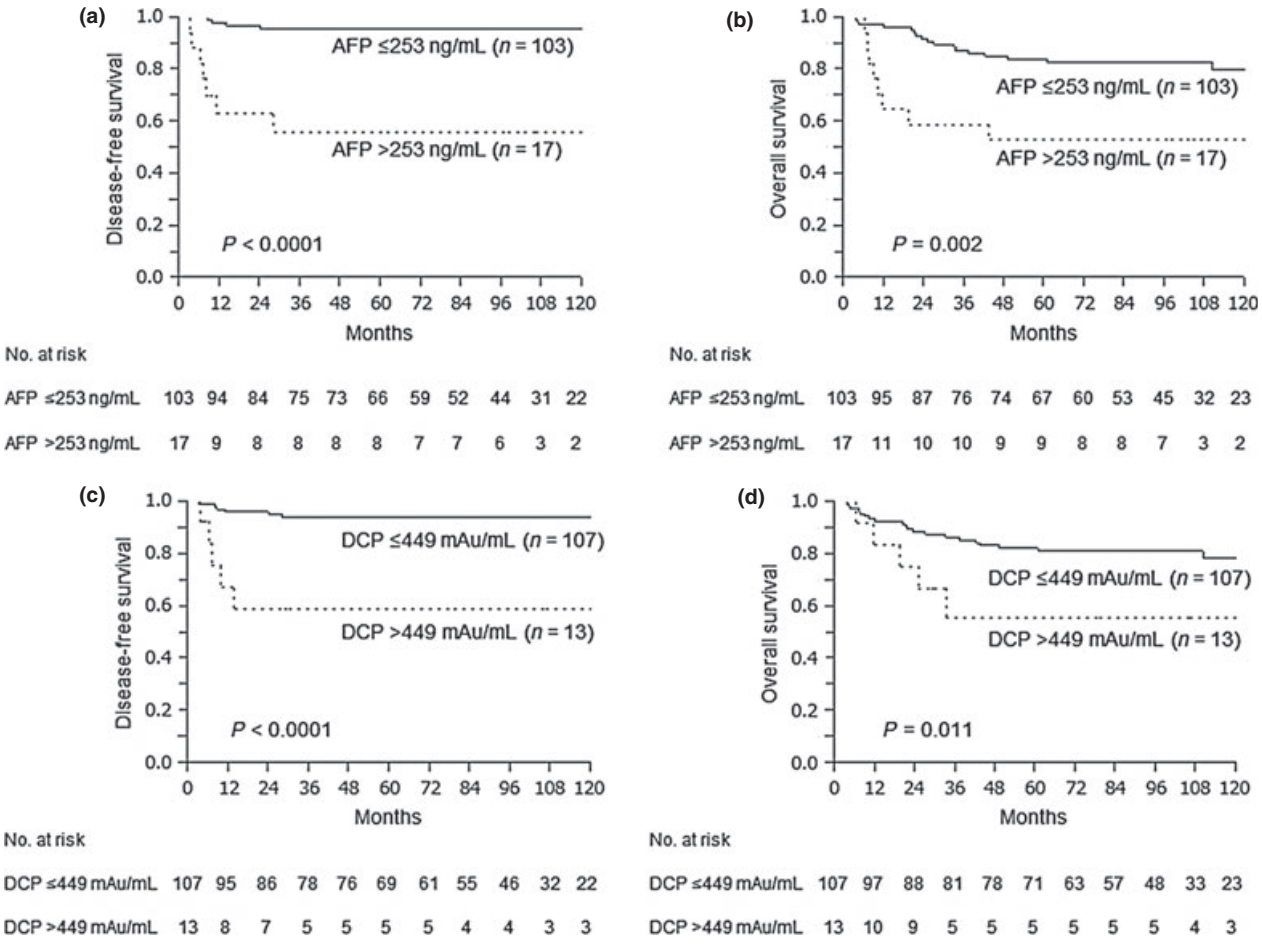

Figure 2 Long-term outcomes according to pretransplant tumor markers. (a,c) Disease-free survival, (b,d) Overall survival. AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin.

tic score was calculated as sum of the three variables [i.e., Tokyo criteria (0 or 1) + Pretransplant maximum AFP >250 ng/ml (0 or 1) + Pretransplant maximum DCP >450 mAu/ml (>450 ng/ml)(0 or 1)]. Significant differences between scores of 0–1 and scores of 2–3 were found in both the 5-year disease-free survival rate (97.9% vs. 20.0%, $P < 0.0001$) and in the 5-year overall survival rate (88.4% vs. 20.0%, $P = 0.0001$; Fig. 3).

Similar results were also observed when UCSF criteria were used. The 5-year disease-free survival rates for score 0–1 and 2–3 were 96.7% vs. 38.5%, respectively ($P < 0.0001$), and the 5-year overall survival rates for score 0–1 and 2–3 were 84.7% and 38.5%, respectively ($P < 0.0001$).

Discussion

In this study, we analyzed 124 patients who underwent LDLT for HCC. We determined that the maximum AFP and DCP values and the mean NLR value were more sensitive for predicting tumor recurrence after transplantation than the conventional one-point preoperative values measured on the day before transplantation. Multivariate analysis confirmed that AFP, DCP, and NLR were correlated with tumor recurrence, and ROC analysis indicated that both AFP and DCP better predict long-term outcomes. Incorporation of these tumor markers with size and number-based Tokyo criteria better stratifies patient prognosis in LT for HCC.

High DCP levels have been considered a strong predictor of recurrence in patients treated with liver resection [19–22], ablation therapy [23,24], or transarterial chemoembolization [25,26]. Reports of a correlation between DCP levels and the incidence of microvascular invasion [27,28] or poor differentiation [29] support these clinical observations. The Kyoto group recently reported that DCP levels can predict the incidence of microvascular invasion in patients undergoing LT [1]. They found that DCP levels

Table 5. Prognostic score.

Score	0	1
Tokyo criteria*	Match	Not match
Pretransplant maximum AFP	≤250 ng/ml	>250 ng/ml
Pretransplant maximum DCP	≤450 mAu/ml	>450 mAu/ml

AFP, alpha-fetoprotein; DCP, des-gamma-carboxyprothrombin.
Prognostic score is calculated as some of the three variables (i.e., Tokyo criteria (0 or 1) + Pretransplant maximum AFP >250 ng/ml (0 or 1) + Pretransplant maximum DCP >450 mAu/ml (0 or 1).
*≤5 tumors with each tumor ≤5 cm.

>400 mAu/ml are a strong prognostic factor. The current analysis indicated similar outcomes using maximum DCP levels within 90 days before transplantation. Also, patients with higher DCP levels (>449 mAu/ml) had a higher incidence of microvascular invasion and tended to have a higher tumor grade.

Regarding AFP, several authors have reported that speed of increases in AFP level (>50 mg/L/month [2] or >15 mg/L/month [5]) can predict tumor recurrence after LT. Because 40% of the patient population in the present study underwent some treatment for HCC prior to transplantation (Table 1), AFP levels decreased during the pretransplant work-up period in most of the cases. Accordingly, it was difficult to evaluate the association of the oncologic activity of the tumor with the progression speed of AFP. Nevertheless, the present study indicated that maximum AFP values within 90 days prior to transplantation could sensitively discriminate patients at high risk of tumor recurrence and poor prognosis. Histopathologic features of the tumors confirmed that higher AFP level was also correlated with aggressive tumor properties, such as a higher incidence of microvascular invasion and poorer differentiation.

In addition to the tumor markers, a possible correlation between preoperative inflammatory status and patient prognosis was recently reported both in surgical resection [30–32] and transplantation studies [6,7,33,34]. Recent studies reported that NLR can be a surrogate marker for the risk of tumor recurrence reflecting the inflammatory tumoral microenvironment [6,7]. Although the clinical relevance and prognostic value of NLR remain controversial, the findings of the present study also confirmed that NLR was correlated with tumor recurrence in multivariate analysis. The ability of NLR to discriminate patients at high risk of tumor recurrence was relatively poor, and in this aspect, NLR was inferior to AFP or DCP (Fig. 1 and Table 3).

Based on these results, we tested the synergic effect of tumor markers on our conventional indication criteria, up to 5 tumors with the diameter of each being no larger than 5 cm (i.e., Tokyo criteria). As shown in Fig. 3, the presence of at least two of the following factors: (i) exceeding the Tokyo criteria, (ii) AFP >250 ng/ml, and (iii) DCP >450 mAu/ml was strongly correlated with worse disease-free survival and overall survival. Good prognosis can be expected with lower AFP and lower DCP, even when a patient has HCC exceeding the Tokyo criteria (score 1), while the prognosis will be very poor when both AFP and DCP levels exceed these criteria, even when a patient has HCC meeting the Tokyo criteria (score 2) both in overall survival and disease-free survival.

Limitations of the present study include its retrospective nature and lack of protocol for the timing of the measurement of tumor markers. This study was based on a prospectively collected database, however, and the solid trend data based on the multiple blood samplings before transplantation allowed us to compare several types of parameters (e.g., maximum value, mean value, and immediate pretransplant value). In addition, because this study lacks a

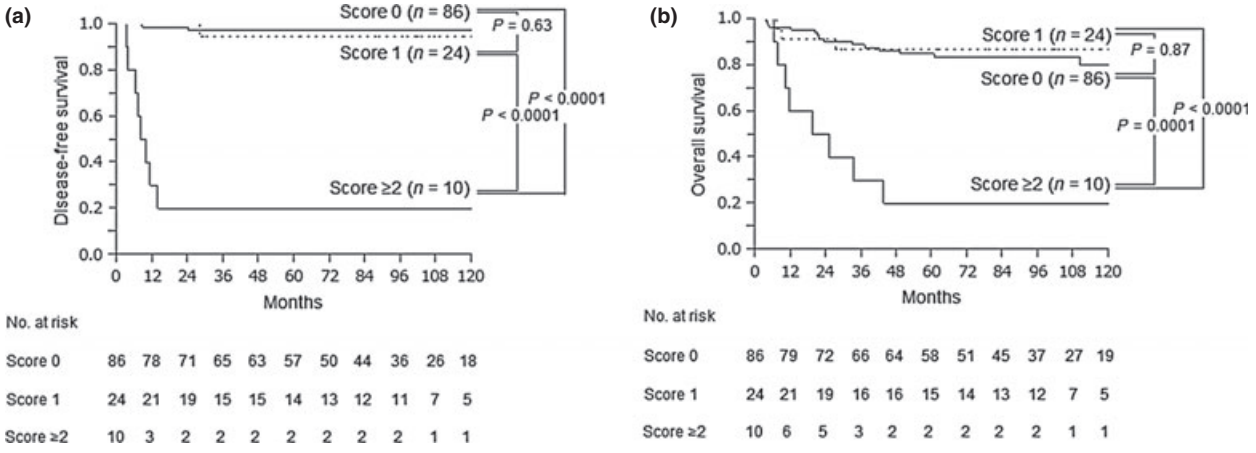

Figure 3 Long-term outcomes based on a new prognostic score system. (a) Disease-free survival, (b) Overall survival.

validation set to confirm the clinical relevance of our new scoring system, a validation study should be performed by other transplant centers.

In conclusion, the prognosis of patients undergoing LT for HCC strongly relies on maximum AFP or DCP values before transplantation, while the prognostic impact of NLR is limited. Incorporation of these tumor markers with conventional indication criteria may better stratify patients with risk of tumor recurrence and poor prognosis.

Authorship

JS and YS: participated in study design. JS, YS, RN, JK, ST, TA, YS, KH, TT, and NK: involved in acquisition of data and writing manuscript. JS, YS, RN: involved in data analysis. YS and NK participated in critical revision.

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References

1. Fujiki M, Takada Y, Ogura Y, *et al.* Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362.
2. Han K, Tzimas GN, Barkun JS, *et al.* Preoperative alpha-fetoprotein slope is predictive of hepatocellular carcinoma recurrence after liver transplantation. *Can J Gastroenterol* 2007; **21**: 39.
3. Shimada M, Yonemura Y, Ijichi H, *et al.* Living donor liver transplantation for hepatocellular carcinoma: a special reference to a preoperative des-gamma-carboxy prothrombin value. *Transplant Proc* 2005; **37**: 1177.
4. Taketomi A, Sanefuji K, Soejima Y, *et al.* Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009; **87**: 531.
5. Vibert E, Azoulay D, Hoti E, *et al.* Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129.
6. Halazun KJ, Hardy MA, Rana AA, *et al.* Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009; **250**: 141.
7. Motomura T, Shirabe K, Mano Y, *et al.* Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol* 2013; **58**: 58.
8. Mano Y, Shirabe K, Yamashita Y, *et al.* Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 2013; **258**: 301.
9. Lai Q, Castro Santa E, Rico Juri JM, Pinheiro RS, Lerut J. Neutrophil and platelet-to-lymphocyte ratio as new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer. *Transplant Int* 2013; **27**: 32.
10. Sapisochin G, Yao FY, Roberts JP. Do preoperative inflammatory markers impact on outcome after liver transplantation for hepatocellular carcinoma? *Ann Surg* 2011; **254**: 383.
11. Lee E, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of alpha-fetoprotein, over time. *Clin Gastroenterol Hepatol* 2013; **11**: 437.
12. Chen TM, Huang PT, Tsai MH, *et al.* Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. *J Gastroenterol Hepatol* 2007; **22**: 669.
13. Deyashiki Y, Nishioka Y, Takahashi K, Kosaka Y, Suzuki K. Evaluation of des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma. *Cancer* 1989; **64**: 2546.
14. Kokudo N, Sugawara Y, Imamura H, Sano K, Makuuchi M. Tailoring the type of donor hepatectomy for adult living donor liver transplantation. *Am J Transplant* 2005; **5**: 1694.
15. Shindoh J, Sugawara Y, Akamatsu N, *et al.* Thrombotic microangiopathy after living-donor liver transplantation. *Am J Transplant* 2012; **12**: 728.
16. Mazzaferro V, Regalia E, Doci R, *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693.
17. Yao FY, Ferrell L, Bass NM, *et al.* Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394.
18. Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25**: 310.
19. Hakamada K, Kimura N, Miura T, *et al.* Des-gamma-carboxy prothrombin as an important prognostic indicator in patients with small hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1370.
20. Kim do Y, Paik YH, Ahn SH, *et al.* PIVKA-II is a useful tumor marker for recurrent hepatocellular carcinoma after surgical resection. *Oncology* 2007; **72**(Suppl. 1): 52.
21. Nanashima A, Morino S, Yamaguchi H, *et al.* Modified CLIP using PIVKA-II for evaluating prognosis after hepatectomy for hepatocellular carcinoma. *Eur J Surg Oncol* 2003; **29**: 735.
22. Shimada M, Takenaka K, Fujiwara Y, *et al.* Des-gamma-carboxy prothrombin and alpha-fetoprotein positive status as a new prognostic indicator after hepatic resection for hepatocellular carcinoma. *Cancer* 1996; **78**: 2094.
23. Kobayashi M, Ikeda K, Kawamura Y, *et al.* High serum des-gamma-carboxy prothrombin level predicts poor prognosis

after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; **115**: 571.
24. Tateishi R, Shiina S, Yoshida H, *et al.* Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; **44**: 1518.
25. Kishi K, Sonomura T, Mitsuzane K, *et al.* Time courses of PIVKA-II and AFP levels after hepatic artery embolization and hepatic artery infusion against hepatocellular carcinoma: relation between the time course and tumor necrosis. *Radiat Med* 1992; **10**: 189.
26. Minakuchi K, Murata K, Kaminoh T, *et al.* Clinical evaluation of serum alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) levels in patients with hepatocellular carcinoma following interventional radiology. *Radiat Med* 1993; **11**: 231.
27. Koike Y, Shiratori Y, Sato S, *et al.* Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. *Cancer* 2001; **91**: 561.
28. Suehiro T, Matsumata T, Itasaka H, Taketomi A, Yamamoto K, Sugimachi K. Des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. *Surgery* 1995; **117**: 682.
29. Okuda H, Nakanishi T, Takatsu K, *et al.* Serum levels of des-gamma-carboxy prothrombin measured using the revised enzyme immunoassay kit with increased sensitivity in relation to clinicopathologic features of solitary hepatocellular carcinoma. *Cancer* 2000; **88**: 544.
30. Gomez D, Farid S, Malik HZ, *et al.* Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. *World J Surg* 2008; **32**: 1757.
31. Chun JM, Kwon HJ, Sohn J, *et al.* Prognostic factors after early recurrence in patients who underwent curative resection for hepatocellular carcinoma. *J Surg Oncol* 2011; **103**: 148.
32. Nishikawa H, Arimoto A, Wakasa T, Kita R, Kimura T, Osaki Y. Pre-treatment C-reactive protein as a prognostic factor for recurrence after surgical resection of hepatocellular carcinoma. *Anticancer Res* 2013; **33**: 1181.
33. Limaye AR, Clark V, Soldevila-Pico C, *et al.* Neutrophil-lymphocyte ratio predicts overall and recurrence-free survival after liver transplantation for hepatocellular carcinoma. *Hepatol Res* 2012; **43**: 757.
34. An HJ, Jang JW, Bae SH, *et al.* Serum C-reactive protein is a useful biomarker for predicting outcomes after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2012; **18**: 1406.