RETROSPECTIVE STUDY

Inflammatory markers as selection criteria of hepatocellular carcinoma in living-donor liver transplantation

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Abstract

AIM: To investigate that inflammatory markers can predict accurately the prognosis of hepatocelluar carcinoma (HCC) patients in living-donor liver transplantation (LDLT).

METHODS: From October 2000 to November 2011, 224 patients who underwent living donor liver transplantation for HCC at our institution were enrolled in this study. We analyzed disease-free survival (DFS) and overall survival (OS) after LT in patients with HCC and designed a new score model using pretransplant neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP).

RESULTS: The DFS and OS in patients with an NLR level ⩾ 6.0 or CRP level ⩾ 1.0 were significantly worse than those of patients with an NLR level < 6.0 or CRP level < 1.0 (*P* = 0.049, *P* = 0.003 for NLR and *P* = 0.010, *P* < 0.001 for CRP, respectively). Using a new score model using the pretransplant NLR and CRP, we can differentiate HCC patients beyond the Milan criteria with a good prognosis from those with a poor prognosis.

CONCLUSION: Combined with the Milan criteria, new score model using NLR and CRP represent new selection criteria for LDLT candidates with HCC, especially beyond the Milan criteria.

Core tip: Although the Milan criteria are accepted as the standard selection criteria for liver transplantation candidates with hepatocelluar carcinoma (HCC), they are so strict; New selection criteria are needed to predict more accurately the prognosis of patients with HCC; Using a new score model using pretransplant neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP), we can differentiate HCC patients beyond the Milan criteria with a good prognosis from those with a poor prognosis; Combined with the Milan criteria, a new score model using pretransplant NLR and CRP may represent new selection criteria for living-donor liver transplantation candidates with HCC, especially beyond the Milan criteria.

INTRODUCTION

Among the several treatment modalities for hepatocellu-

lar carcinoma (HCC), liver transplantation (LT) and surgical resection are curative methods. Chronic liver disease is one of the main etiologies of HCC; approximately 80% of patients with HCC have cirrhosis[1]. LT is an ideal treatment for selected patients with HCC because it targets not only the tumor but also the underlying liver disease[2]. Since the introduction of the Milan criteria by Mazzaferro *et al*[3] in 1996, disease-free survival and overall survival after LT for patients with HCC meeting the Milan criteria have been equivalent to those of non-HCC patients. Although survival rates after LT have improved dramatically, HCC recurrence remains a significant problem. It has been demonstrated that about 10% of HCC patients within the Milan criteria experience HCC recurrence[4]. In contrast, some patients with HCC beyond the Milan criteria may have favorable outcomes[5]. There are some limitations to the Milan criteria that should be addressed in any new standard selection criteria for HCC patients. Inflammation is a critical component of tumor progression[6]. Several inflammatory markers, such as the neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP), have been suggested as surrogate markers of treatment response and survival in patients with HCC[7-9]. However, few studies have examined the relationship between these factors and HCC recurrence after living-donor liver transplantation (LDLT).

The aim of the present study was to assess whether the pretransplant NLR and CRP levels can accurately predict disease-free survival and overall survival after LDLT in patients with HCC. Furthermore, we established a pretransplant score model that may assist in the selection of patients with HCC that would benefit from liver transplantation.

MATERIALS AND METHODS

Patients
From October 2000 to November 2011, a total of 243 patients underwent LDLT for HCC at our transplant center. Nineteen patients were excluded from the study: 7 for postoperative mortality within 30 d after transplantation, 6 for preoperative infection, 4 for undergoing pretransplant locoregional treatment within 1 mo before transplantation, and 2 for massive alimentary tract bleeding within 1 mo before transplantation. After excluding these nineteen cases, the medical records of 224 patients were reviewed retrospectively. This study was approved by the Institutional Review Board of our center.

Diagnosis and treatment strategy of HCC
All patients who were to undergo transplantation for HCC were evaluated preoperatively by dynamic liver computed tomography (CT) and enhanced magnetic resonance imaging (MRI). Chest CT, bone scan, and positron emission tomography CT (PET CT) were performed to exclude distant metastasis and other primary malignancies. Contraindications for LT in patients with HCC included a tumor thrombus in the main portal vein,

regional lymph node metastasis, and distant metastasis. The alpha-fetoprotein (AFP) level and the proteins induced by vitamin K absence or angiotensin-Ⅱ (PIVKA-Ⅱ) level were evaluated as tumor markers. Hepatitis viral markers and liver function tests were also assessed. Neutrophil and lymphocyte counts were routinely measured on the day before transplantation, with the NLR calculated by dividing the neutrophil count by the lymphocyte count. The serum CRP level was measured with a turbidimetric immunoassay (Wako Chemicals GmbH, Neuss, Germany). The pretransplant CRP level was not measured routinely until 2006 and then was checked routinely on the day before transplantation. When HCC was diagnosed, the treatment was based on the tumor stage and the patient's liver function. Patients who were eligible for transplantation underwent DDLT or LDLT according to the meeting the Milan criteria and the availability of liver donor. Pretransplant locoregional treatments include hepatic resection, transarterial chemoembolization (TACE), radiofrequency ablation, and percutaneous ethanol injection, which were selected according to the Barcelona Clinic Liver Cancer scoring system. TACE was the primary treatment modality among pretransplant locoregional treatment modalities.

Liver transplantation and post-transplant follow-up
LDLT was performed according to a standard technique using a modified right lobe with middle hepatic vein reconstruction. For patients with ascites, aspiration and cytology were performed before beginning the operation. When lymph node enlargement was present, or in cases with suspicious metastatic disease, an intraoperative biopsy was performed. The operation was performed only in cases with negative biopsy results.

Immunosuppression regimens consisted of a triple drug regimen that included tacrolimus or cyclosporin, mycophenolatemofetil (MMF), and prednisolone. The dose of tacrolimus was adjusted to maintain levels of 7-10 ng/mL for the first postoperative month and 5-7 ng/mL thereafter. The dose of cyclosporin was adjusted to maintain levels of 100-150 ng/mL for the first postoperative month and 50-100 ng/mL thereafter. Steroids were withdrawn 1 mo after surgery, and MMF was withdrawn 6 mo after surgery. An interleukin-2 receptor blocker was administered on both the day of surgery and the fourth postoperative day.

Patients were followed weekly after hospital discharge until they were stable and then monthly for the first year, every 2 mo for 5 years, and then every 3 mo. Tumor markers were measured monthly during the first year, and then every 2 mo thereafter. Abdomen CT, chest CT, and bone scintigraphy were routinely performed every 4 mo for the first year, every 6 mo for the next year, and then annually. When tumor recurrence was suspected, MRI and/or PET-CT were performed.

Statistical analysis
Continuous variables are reported as mean ± SD and

Table 1	Clinical parameters of the study population n (%)
Parameter	**Value**
Mean age (yr)[1]	51.9 ± 6.9
Male	184 (82.1)
Diagnosis	
Hepatitis B	197 (87.9)
Hepatitis C	13 (5.8)
Others	14 (6.3)
GRWR[1]	1.21 ± 0.27
Child pugh score[1]	8.2 ± 2.4
MELD score[1]	12.8 ± 7.6
AFP (ng/mL)[1]	183.4 ± 762.7
PIVKA-II (mAU/mL)[1]	206.5 ± 1118.7
C-reactive protein (mg/dL)[1]	1.36 ± 2.74
Neutrophil lymphocyte ratio[1]	3.47 ± 4.68
Pretransplant treatment for HCC	167 (74.6)
Tumor number[1]	2.6 ± 2.4
Maximum tumor size (cm)[1]	3.2 ± 3.1
Microvascular invasion	44 (21.3)
Edmondson-steiner grade (III-IV)	81 (42.9)
Within the milan criteria	133 (59.4)
Follow-up duration (mo)[1]	48.9 ± 37.3
	(median: 68, range: 6-139)

[1]Values shown are mean ± SD except where stated otherwise. GRWR: Graft-to-recipient body weight ratio; MELD: Model for end-stage liver disease; AFP: Alpha-fetoprotein; PIVKA-II: Proteins induced by vitamin K antagonism or absence-II; HCC: Hepatocellular carcinoma.

were compared using the Student t test. Categorical variables were analyzed using the χ^2 test. To evaluate the risk factors for HCC recurrence, univariate analysis of risk factors was performed using the Kaplan-Meier method and evaluated using the log-rank test. Candidate predictors associated with a P value less than 0.2 on univariate analysis were entered into a multivariate analysis using Cox regression analysis. The CRP level was excluded from the multivariate analysis because the number of patients with an available CRP level was small compared with other clinico-pathological variables. Subgroup analysis by the Milan criteria was also conducted. Overall survival and disease-free survival were calculated using the Kaplan-Meier method and evaluated with the log-rank test. Statistical analysis was performed using SPSS (Chicago, IL, United States) 18.0 for Windows. A P value < 0.05 was considered to indicate statistical significance.

RESULTS

Patients' characteristics

Among the 224 patients, 184 (82.1%) were male, and the mean age was 51.9 ± 6.9 years. The most common cause for LT was hepatitis B (n = 197, 87.9%), followed by hepatitis C (n = 13, 5.8%) and other causes (n = 14, 6.3%). Pretransplant locoregional treatments for HCC were performed in 167 patients (74.6%). Of the 224 patients, 133 (59.4%) met the Milan criteria. The mean Child-Pugh score was 8.2 ± 2.4, and the mean Model for End-stage Liver Disease (MELD) score was 12.8 ± 7.6 (Table 1).

The median follow-up period was 68 mo (range, 6-139 mo). The 1, 3, and 5 year overall survival rates were

Table 2	Disease-free survival and overall survival according to neutrophil-lymphocyte ratio and C-reactive protein			
	Disease-free survival		**Overall survival**	
	χ^2	***P* value**	χ^2	***P* value**
Neutrophil-lymphocyte ratio (n = 224)				
NLR ≥ 1 (n = 195)	1.041	0.308	0.125	0.724
NLR ≥ 2 (n = 115)	2.938	0.087	2.777	0.096
NLR ≥ 3 (n = 70)	0.746	0.388	3.308	0.069
NLR ≥ 4 (n = 44)	1.132	0.287	5.301	0.021
NLR ≥ 5 (n = 34)	2.383	0.123	7.257	0.007
NLR ≥ 6 (n = 27)	3.497	0.049	8.799	0.003
NLR ≥ 7 (n = 22)	1.379	0.240	6.411	0.001
C-reactive protein (n = 145)				
CRP ≥ 0.5 (n = 72)	1.359	0.244	4.032	0.045
CRP ≥ 1.0 (n = 42)	6.653	0.010	12.604	< 0.001
CRP ≥ 2.0 (n = 25)	6.974	0.008	6.728	0.009

NLR: Neutrophil-lymphocyte ratio; CRP: C-reactive protein.

88.5%, 78.0% and 76.6%, respectively. During the follow-up period, 50 patients (22.3%) died. The cause of death was HCC recurrence in 31 patients (62.0%), technical complications in nine patients (18.0%), sepsis in five patients (10.0%), graft failure in three patients (6.0%), and other causes in two patients (4.0%). The 1, 3, and 5 year disease-free survival rates were 88.3%, 83.3% and 81.6%, respectively. Most HCC recurrences (n = 30, 81.1%) occurred within 2 years, with 26 patients (70.3%) experiencing HCC recurrence within 1 year. Two patients (5.4%) experienced HCC recurrence 5 years after transplantation.

Correlation between NLR and HCC recurrence after LDLT

To determine whether an elevated NLR level was correlated with HCC recurrence after LDLT, we performed the Kaplan-Meier analysis with the log-rank test. Using NLR cut-offs of 1, 2, 3, 4, 5, 6 and 7 and comparing disease-free survival and overall survival rates, we showed that an NLR of 6 was the most significant, with a χ^2 value of 3.497 and a P value of 0.049 for disease-free survival and a χ^2 value of 8.799 and a p value of 0.003 for overall survival (Table 2). Of the 224 patents, 27 (12.1%) had an NLR ≥ 6.0. Total bilirubin level (P = 0.006), Child-Pugh score (P < 0.001), MELD score (P < 0.001), and CRP level (P = 0.035) were significantly different between the patients with an NLR level ≥ 6.0 and those with an NLR level < 6.0. Tumor number and maximum tumor size, tumor biologic factors such as microvascular invasion and tumor grade, and tumor markers were not significantly different between the two groups of patients. Also, the NLR level was significantly correlated with the total bilirubin level (r = 0.384, P < 0.001), Child-Pugh score (r = 0.268, P < 0.001), MELD score (r = 0.419, P < 0.001), and CRP level (r = 0.220, P = 0.008) (Table 3).

Correlation between CRP and HCC recurrence after LDLT

To determine whether the elevated CRP level was correlated with HCC recurrence after LDLT, we performed

Table 3 Clinicopathological characteristics according to Neutrophil-lymphocyte ratio and C-reactive protein *n* (%)

Parameters	NLR < 6 (*n* = 197)	NLR ≥ 6 (*n* = 27)	*P* value	CRP < 1 (*n* = 103)	CRP ≥ 1 (*n* = 42)	*P* value
Mean age (yr)[1]	51.8 ± 7.0	52.2 ± 6.3	0.748	52.4 ± 6.9	52.4 ± 6.8	0.992
Male	164 (83.2)	20 (74.1)	0.243	81 (78.6)	35 (83.3)	0.522
Etiology (HBV)	172 (87.3)	25 (92.6)	0.345	90 (87.4)	37 (88.1)	0.991
GRWR[1]	1.20 ± 0.27	1.24 ± 0.30	0.495	1.21 ± 0.29	1.23 ± 0.26	0.777
Total bilirubin (g/dL)[1]	3.8 ± 6.8	11.1 ± 12.5	0.006	3.2 ± 5.8	11.5 ± 12.2	< 0.001
PT INR[1]	2.6 ± 15.3	1.7 ± 0.5	0.771	1.4 ± 0.3	6.6 ± 33.2	0.293
Child Pugh score[1]	7.9 ± 2.3	9.8 ± 2.2	< 0.001	7.8 ± 2.4	9.9 ± 1.9	< 0.001
MELD score[1]	11.7 ± 6.8	20.6 ± 8.8	< 0.001	10.9 ± 6.0	19.4 ± 8.9	< 0.001
AFP (ng/mL)[1]	142 ± 366	490 ± 1995	0.383	128 ± 381	346 ± 1593	0.389
NLR[1]	2.12 ± 1.19	13.3 ± 7.79	< 0.001	2.54 ± 2.71	6.15 ± 7.37	0.003
CRP (mg/dL)[1]	1.03 ± 2.13	3.55 ± 4.76	0.035	0.33 ± 0.29	3.87 ± 4.13	< 0.001
Tumor number[1]	2.5 ± 2.3	3.3 ± 2.8	0.162	2.7 ± 2.4	2.4 ± 2.1	0.352
Maximal tumor size[1]	3.10 ± 2.37	4.03 ± 6.13	0.441	2.9 ± 1.5	4.1 ± 5.8	0.475
Microvascular invasion (+)	38 (21.0)	6 (23.1)	0.800	20 (21.1)	6 (15.0)	0.225
E-S grade (Ⅲ-Ⅳ)	94 (57.3)	14 (56.0)	0.901	38 (44.7)	17 (45.9)	0.415
Beyond the Milan criteria	69 (36.5)	14 (51.9)	0.125	39 (39.0)	15 (36.6)	0.899
Pretransplant locoregional treatment	146 (74.1)	21 (77.8)	0.682	81 (78.6)	30 (71.4)	0.352

[1]Values shown are mean ± SD. NLR: Neutrophil-lymphocyte ratio; CRP: C-reactive protein; HBV: Hepatitis B virus; GRWR: Graft-to-recipient body weight ratio; PT INR: Prothrombin time international normalized ratio; MELD: Model for end-stage liver disease; AFP: Alpha-fetoprotein; E-S grade: Edmondson grade.

the Kaplan-Meier analysis with the log-rank test. Using CRP cut-offs of 0.5, 1.0 and 2.0 and comparing disease-free survival and overall survival rates, we showed that a CRP of 1.0 was the most significant, with a χ^2 value of 6.653 and a *P* value of 0.010 for disease-free survival and a χ^2 value of 12.604 and a *P* value of less than 0.001 for overall survival (Table 2). Of the 145 patents, 42 (29.0%) had a CRP ≥ 1.0. Total bilirubin level (*P* < 0.001), Child-Pugh score (*P* < 0.001), MELD score (*P* < 0.001), and NLR level (*P* = 0.003) were significantly different between patients with a CRP level ≥ 1.0 and those with a CRP level < 1.0, as with the results for NLR. Tumor number and maximum tumor size, tumor biologic factors such as microvascular invasion and tumor grade, and tumor markers were not significantly different between the two groups of patients. Also, the CRP level was significantly correlated with the total bilirubin level (*r* = 0.207, *P* = 0.012), Child-Pugh score (*r* = 0.216, *P* = 0.009), MELD score (*r* = 0.272, *P* = 0.001), the tumor number (*r* = 0.415, *P* < 0.001), and the NLR level (*r* = 0.220, *P* = 0.008) (Table 3).

Prognostic factors for disease free survival and overall survival after LDLT

On univariate analysis, the NLR level ≥ 6.0 (*P* = 0.049), CRP level ≥ 1.0 (*P* = 0.010), AFP ≥ 100 (*P* = 0.015), pretransplant locoregional treatment (*P* = 0.017), tumor size ≥ 5 cm (*P* < 0.001), and microvascular invasion (*P* = 0.024) were significantly associated with HCC recurrence after LDLT. According to multivariate analysis, AFP ≥ 100 (HR= 2.588, 95%CI: 1.187-5.645, *P* = 0.017) and tumor size ≥ 5 cm (HR = 6.014; 95%CI: 2.432-14.869, *P* < 0.001) were independent risk factors for HCC recurrence after LDLT. An NLR level ≥ 6.0 was a significant risk factor for tumor recurrence with marginal significance

(HR = 2.512; 95%CI: 0.987-6.391, *P* = 0.053). Also, an NLR level ≥ 6.0 (*P* = 0.003), CRP level ≥ 1.0 (*P* < 0.001), AFP ≥ 100 (*P* = 0.048), pretransplant locoregional treatment (*P* = 0.023), and tumor size ≥ 5 cm (*P* = 0.001) were significantly associated with overall survival after LDLT on univariate analysis. An NLR level ≥ 6.0 was an only independent prognostic factor for poor survival on multivariate analysis (HR = 2.896; 95%CI: 1.399-5.996, *P* = 0.004) (Table 4).

We analyzed disease-free survival and overall survival for patients according to the Milan criteria. For 133 patients (59.4%) within the Milan criteria, an NLR level ≥ 6.0 was associated with decreased overall survival (*P* = 0.037) but was not associated with disease-free survival (*P* = 0.541). A CRP level ≥ 1.0 was not associated with disease-free survival (*P* = 0.797) but was associated with decreased overall survival, but with only marginal significance (*P* = 0.054). A tumor size ≥ 5 cm was an only independent prognostic factor for HCC recurrence on multivariate analysis (HR = 6.980; 95%CI: 1.497-32.535), *P* = 0.013) (Table 5).

For 91 patients (40.6%) beyond the Milan criteria, NLR level ≥ 6.0 (HR = 3.973; 95%CI: 1.288-12.249, *P* = 0.016) and AFP ≥ 100 (HR = 3.619; 95%CI: 1.184-11.063, *P* = 0.024) were independent risk factors for HCC recurrence after LDLT. An NLR level ≥ 6.0 was an only independent prognostic factor for poor survival on multivariate analysis (HR = 4.685; 95%CI: 1.607-13.657, *P* = 0.005). A CRP level ≥ 1.0 was significantly associated with both disease-free survival (*P* = 0.004) and overall survival (*P* = 0.001) on univariate analysis (Table 5).

Pretransplant prognostic score model using NLR and CRP

We established a pretransplant new prognostic factor

Table 4 Prognostic factors for disease free survival and overall survival in the whole study population

	Disease free survival			Overall survival		
	Univariate	Multivariate analysis		Univariate	Multivariate analysis	
	P value	HR (95%CI)	*P* value	*P* value	HR (95%CI)	*P* value
Age ⩾ 60	0.404			0.723		
Male gender	0.196	2.086 (0.595-7.307)	0.250	0.411		
Etiology (HBV)	0.977			0.775		
GRWR ⩾ 1.0	0.551			0.639		
Child C	0.978			0.810		
AFP ⩾ 100	0.015	2.588 (1.187-5.645)	0.017	0.048	1.567 (0.822-2.987)	0.172
NLR ⩾ 6	0.049	2.512 (0.987-6.391)	0.053	0.003	2.896 (1.399-5.996)	0.004
Pretransplant locoregional treatment	0.017	4.604 (1.074-19.740)	0.040	0.023	1.946 (0.804-4.713)	0.140
Tumor number ⩾ 2	0.604			0.913		
Tumor size ⩾ 5 cm	< 0.001	6.014 (2.432-14.869)	< 0.001	0.001	2.206 (0.968-5.028)	0.060
Microvascular invasion (+)	0.024	1.369 (0.599-3.132)	0.456	0.074	1.554 (0.769-3.141)	0.220
E-S grade (Ⅲ-Ⅳ)	0.172	1.040 (0.470-2.302)	0.923	0.082	1.371 (0.715-2.630)	0.342
CRP ⩾ 1.0 (*n* = 145)	0.010			< 0.001		

HBV: Hepatitis B virus; GRWR: Graft-to-recipient body weight ratio; AFP: Alpha-fetoprotein; NLR: Neutrophil lymphocyte ratio; E-S grade: Edmondson grade; CRP: C-reactive protein.

Table 5 Prognostic factors for disease free survival and overall survival in patients with hepatocelluar carcinoma according to the Milan criteria

	Disease free survival			Overall survival		
	Univariate	Multivariate analysis		Univariate	Multivariate analysis	
	P value	HR (95%CI)	*P* value	*P* value	HR (95%CI)	*P* value
Within the Milan criteria						
AFP ⩾ 100	0.475			0.471		
NLR ⩾ 6	0.541			0.037	2.509 (0.945-6.658)	0.065
Pretransplant locoregional treatment	0.193	2.126 (0.587-7.710)	0.251	0.257		
Tumor number ⩾ 2	0.764			0.459		
Tumor size ⩾ 5 cm	< 0.001	6.980 (1.497-32.535)	0.013	0.681		
Microvascular invasion (+)	0.619			0.307		
E-S grade (Ⅲ-Ⅳ)	0.835			0.592		
CRP ⩾ 1.0 (*n* = 145)	0.797			0.054		
Beyond the Milan criteria						
AFP ⩾ 100	0.058	3.619 (1.184-11.063)	0.024	0.082	2.456 (0.888-6.793)	0.083
NLR ⩾ 6	0.034	3.973 (1.288-12.249)	0.016	0.045	4.685 (1.607-13.657)	0.005
Pretransplant locoregional treatment	0.096		0.972	0.086		0.971
Tumor number ⩾ 2	0.001	0.429 (0.108-1.701)	0.228	0.016	0.493 (0.121-2.001)	0.322
Tumor size ⩾ 5 cm	< 0.001	3.753 (0.901-15.626)	0.069	0.003	2.354 (0.587-9.449)	0.227
Microvascular invasion (+)	0.226			0.318		
E-S grade (Ⅲ-Ⅳ)	0.083	2.191 (0.697-6.884)	0.179	0.067	2.618 (0.895-7.651)	0.079
CRP ⩾ 1.0 (*n* = 145)	0.004			0.001		

HBV: Hepatitis B virus; GRWR: Graft-to-recipient body weight ratio; AFP: Alpha-fetoprotein; NLR: Neutrophil lymphocyte ratio; E-S grade: Edmondson grade; CRP: C-reactive protein.

score model based on the results for NLR and CRP. Each factor (NLR level ⩾ 6.0 or CRP level ⩾ 1.0) was given a score of 1, after which patients were divided into three groups according to prognostic scores: those with NLR level < 6.0 and CRP level < 1.0 [score 0, *n* = 97 (66.9%)], those with NLR level ⩾ 6.0 or CRP level ⩾ 1.0 [score 1, *n* = 35 (24.1%)], and those with NLR level ⩾ 6.0 and CRP level ⩾ 1.0 [score 2, *n* = 13 (9.0%)]. The disease-free survival for patients with a score of 1 or 2 was significantly lower than that for patients with a score of 0 (*P* = 0.045 and *P* = 0.006, respectively). The overall survival for patients with a score of 1 or 2 was also significantly lower than that for patients with a score of 0 (*P* = 0.001 and *P* = 0.002, respectively). For patients meeting the Milan criteria, the disease-free survival and the overall survival were not significantly different according to the prognostic score model using NLR and CRP. For patients beyond the Milan criteria, the disease-free survival and the overall survival were significantly superior in patients with a score of 0 compared to those in patients with a score of 2 (*P* = 0.004 and *P* = 0.001, respectively) (Figure 1).

DISCUSSION

LT is considered an ideal treatment for selected patients with HCC because it can treat not only the tumor but also the underlying liver disease. Since the introduction
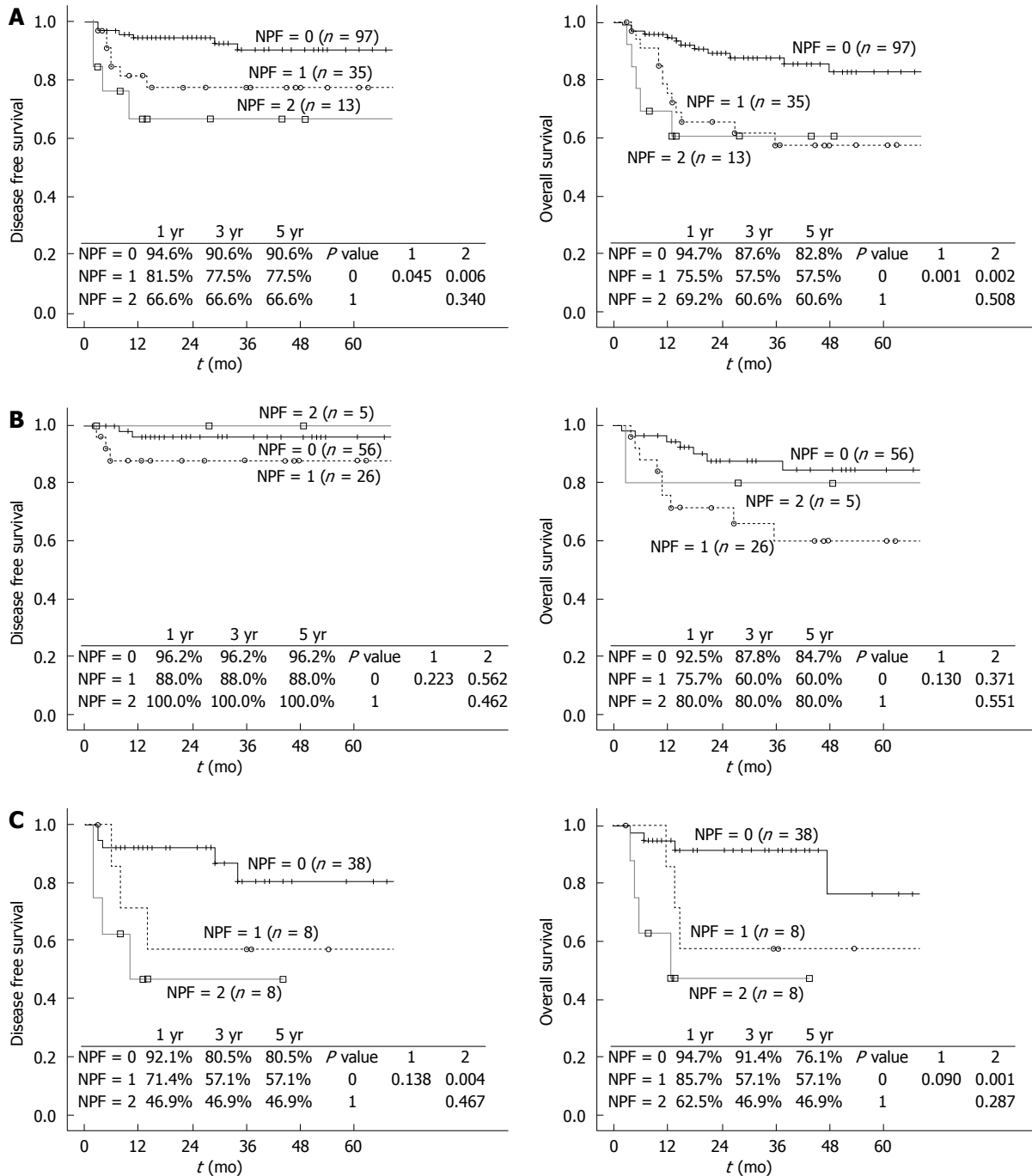
Figure 1 Disease free survival and overall survival according to the new prognostic factor score model. Based on the results for neutrophil-lymphocyte ratio and C-reactive protein; each factor (neutrophil-lymphocyte ratio level ≥ 6.0 or CRP level ≥ 1.0) was given a score of 1. A: Whole population; B: Within the Milan criteria; C: Beyond the Milan criteria. NPF: New prognostic factor.

of the Milan criteria by Mazzaferro *et al*[3] in 1996, the disease-free survival and overall survival after LT for patients with HCC meeting the Milan criteria have been equivalent to those of non-HCC patients. LT has become the treatment of choice for cirrhotic patients with HCC. However, there are some limitations in the Milan criteria that must be adopted in the standard selection criteria for HCC patients. First, the Milan criteria depend exclusively on tumor size and number of pretransplant radiologic evaluations. Many reports have demonstrated that tumor biology such as microvascular invasion and tumor dif-

ferentiation[10,11] and pretransplant tumor markers such as AFP and PIVKA-Ⅱ[12,13] affect HCC recurrence after LT. Second, because the Milan criteria are so strict, many patients who may benefit from LT will be rejected. Many centers have reported good results despite expansion of the selection criteria[14,15]. Therefore, it is important to establish more appropriate selection criteria for LT candidates with HCC. The aim of the present study was to identify new factors related to DFS and OS after LT in order to expand the selection criteria in patients with HCC.

Inflammation is a critical component of tumor progression[6,16]. Many cancers generate from sites of infection, chronic irritation and inflammation. This process is thought to be related to upregulation of cytokines and inflammatory mediators. Inflammation promotes the proliferation and survival of tumor cells and angiogenesis and provides a good microenvironment for tumor growth[17]. It is reported that proinflammatory cytokines such as TNF, IL-1, and IL-6 as well as tumor associated macrophages and IL-17 are involved in this inflammatory process[18,19]. However, it is difficult to measure these cytokines and factors routinely before transplantation. NLR and CRP have been suggested as surrogate markers which can be easily measured preoperatively. An elevated NLR level is associated with poor outcomes in patients with several types of malignant tumors, including colorectal cancer[20], pancreatic cancer[21], intrahepatic cholangiocarcinoma[22], and HCC[7]. Furthermore, an elevated NLR is related to a poor prognosis in patients undergoing LT for HCC[23,24]. Also, a high CRP level has been shown to be significantly correlated with a poor outcome in patients undergoing hepatic resection for HCC[25,26].

In the present study, the pretransplant NLR and CRP level were predictive of disease-free survival and overall survival after LT in patients with HCC. These factors correlated with hepatic functional parameters such as the MELD score and Child-Pugh score rather than tumor size and number, and were more relevant to overall survival than disease-free survival. Also, the subgroup analysis using the Milan criteria showed that there were more significant differences in disease-free survival and overall survival of patients with HCC beyond the Milan criteria. Because the Milan criteria that adopted the standard selection criteria for HCC patients are so strict, many patients are excluded from LT who could actually benefit greatly from the transplantation. However, the new score model used in this study may help to identify patients with HCC who could benefit most from LT, beyond the limitations imposed by the Milan criteria. The present study is the first report to analyze NLR and CRP levels together and to compare both factors. A new score model using these factors may help to predict disease-free survival and overall survival after LDLT in patients with HCC. Our findings showed that the CRP level was more powerful than the NLR level in predicting disease-free survival and overall survival after LDLT in patients with HCC. However, in the present study, the sample size for the CRP level was smaller than that for the NLR level. If the sample size for the CRP level was increased, better results may be obtained.

There were some limitations to this study. First, it was of a retrospective design. The pretransplant CRP level was not measured routinely until 2006. Thus, the sample size available for the pretransplant CRP level was smaller than the sample sizes for the other data. Second, pretransplant NLR and CRP levels may have been affected by various clinical factors (including preoperative sepsis, recent pretransplant locoregional treatments, and massive alimentary bleeding). We attempted to reduce these confounding factors that can result in falsely elevated NLR and CRP through careful pretransplant examination.

In conclusion, pretransplant NLR and CRP levels are useful biomarkers to predict disease-free survival and overall survival after LDLT in patients with HCC. These factors are more useful in patients with HCC beyond the Milan criteria than in patients with HCC who meet the Milan criteria. Our score model may assist in the selection of patients with HCC who would benefit from LDLT, but would otherwise have been excluded by the Milan criteria.

COMMENTS

Background
Liver transplantation (LT) is an ideal treatment for selected patients with hepatocellular carcinoma (HCC) because it targets not only the tumor but also the underlying liver disease. Although survival rates after LT have improved dramatically, HCC recurrence remains a significant problem. Inflammation is a critical component of tumor progression. Several inflammatory markers, such as the neutrophil-lymphocyte ratio (NLR) and C-reactive protein (CRP), have been suggested as surrogate markers of treatment response and survival in patients with HCC.

Research frontiers
Because the Milan criteria that adopted the standard selection criteria for HCC patients are so strict, many patients are excluded from LT who could actually benefit greatly from the transplantation. The aim of the present study was to identify new factors related to disease-free survival and overall survival after LT in order to expand the selection criteria in patients with HCC.

Innovations and breakthroughs
In the present study, the pretransplant NLR and CRP level were predictive of disease-free survival and overall survival after LT in patients with HCC. Also, the subgroup analysis using the Milan criteria showed that there were more significant differences in disease-free survival and overall survival of patients with HCC beyond the Milan criteria.

Applications
The score model of this paper may assist in the selection of patients with HCC who would benefit from living donor liver transplantation, but would otherwise have been excluded by the Milan criteria.

Peer review
This is a retrospective study including 224 living related LTx for HCC with almost 60% meeting the Milan criteria for LTx. The main finding of the study is that by adding some markers of inflammation such as NLR and CPR there is evidence that the Milan criteria may improve. The authors have used statistical methods trying to find cutoffs for NLR and CRP with reliable results. The authors acknowledge the limitations of their study but the results are of particular interest if reproduced in a prospective way.

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