ORIGINAL ARTICLE

Inflammation-Based Scores Do Not Predict Post-transplant Recurrence of Hepatocellular Carcinoma in Patients Within Milan Criteria

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Increased preoperative inflammation scores, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and inflammation-based index (IBI) have been related to post-transplant HCC recurrence. We evaluated the association between inflammation-based scores (NLR, PLR, IBI) and post-LT HCC recurrence as well as tumor necrosis after transarterial embolization. 150 consecutive patients who underwent transplantation for HCC within the Milan criteria between 1996 and 2010 were included; data regarding inflammatory markers, patient and tumor characteristics were analyzed. NLR, PLR, and IBI were not significantly associated with post-LT HCC recurrence or worse overall survival. Increased NLR and PLR were associated with complete tumor necrosis in the subset of patients who received preoperative transarterial embolization ($P < 0.05$). Cox regression analysis revealed that absence of neoadjuvant transarterial therapy (OR = 4.33, 95% CI = 1.28-14.64; $P = 0.02$) and no fulfillment of the Milan criteria in the explanted liver (OR = 3.34, 95% CI = 1.08-10.35; $P = 0.04$) were independently associated with post-LT HCC recurrence inflammation-based scores did not predict HCC recurrence post-LT in our group of patients. NLR and PLR were associated with better response to TAE, as this was recorded histologically in the explanted liver. Histological fulfillment of the Milan criteria and absence of neoadjuvant transarterial treatment were significantly associated with post-LT HCC recurrence. ***Liver Transpl 20:1327-1335, 2014***. © **2014 AASLD.**

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Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer and the third cause of cancer-related mortality worldwide.[1] Current management depends on the HCC stage at diagnosis and consists of hepatic resection, liver transplantation (LT), radiofrequency ablation, transarterial embolization (TAE)/transarterial chemoembolization (TACE), or systemic therapy.[2] Patients with tumors within the Milan criteria, ie, up to 3 lesions of $\leq$3 cm or a solitary lesion of maximum diameter 5 cm, are potential candidates for LT.[3] Currently, 14% of liver transplants in the United Kingdom are performed as treatment for HCC.[4]

Tumor recurrence is an unfavorable outcome that occurs in approximately 20% of transplanted

Abbreviations: AFP, α-fetoprotein; CI, confidence interval; CRP, c-reactive protein; CT, computed tomography; CTP, Child-Pugh score; CRP, C-reactive protein; GPS, Glasgow prognostic score; HCC, hepatocellular carcinoma; IBI, inflammation-based index; LT, liver transplantation; MRI, magnetic resonance imaging; NLR, neutrophil to lymphocyte ratio; OR, odds ratio; PLR, platelet to lymphocyte ratio; PVA, polyvinyl alcohol; TACE, transarterial chemoembolization; TAE, transarterial embolization.

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patients.[5] The main limitation of Milan criteria is that currently they use a solely radiological evaluation of tumor size and number and do not take into account tumor biological or immunological factors. The latter 2 factors, if validated as preoperative risk factors for cancer relapse, would help to refine the selection of appropriate LT candidates.

Predictive factors of recurrence are currently not well established. Summation of all nodule diameters or total volume of nodules, the absence of neoadjuvant transarterial therapy, the level of α-fetoprotein (AFP), and reduced exposure to calcineurin inhibitors in the first month post LT are some of the factors related to HCC recurrence, but these are not universally reproducible.[6-9] Chronic systemic inflammation has been linked with several human cancers, including HCC, and is associated with poor outcome.[10] Proposed inflammatory scores, such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), inflammation-based index (IBI), and Glasgow prognostic score (GPS), are associated with survival in HCC[11,12] and other cancers.[11,13,14] These scores are increased in patients with advanced HCC and are predictors of poor prognosis.[15] Moreover, they have been associated with high risk of tumor recurrence in colorectal, breast, and other malignancies.[14] The aim of this study was to evaluate the association of NLR, PLR, and IBI with posttransplant HCC recurrence in consecutive patients transplanted for HCC within Milan criteria, based on radiological imaging.

PATIENTS AND METHODS

Patients

One hundred fifty consecutive patients from our prospectively compiled database who underwent liver transplantation for HCCs in the Royal Free Hospital between 1996 and 2010 were included in the study, as previously described.[9] Patients were listed if they were within the Milan criteria based on radiological imaging. Incidental HCCs in the explanted liver were also included in the study (n = 19). All patients after 2001 were offered TAE as a bridging treatment pre-LT if there were no contraindications and on an individual basis before this time, based on physicians' preference. TAE was contraindicated in patients with advanced liver disease (Child-Pugh score more than 8) and/or clinically evident ascites. TAE was performed with selective angiography, with a subsequent embolization using polyvinyl alcohol (PVA) particles as the embolizing agent. The diameter of PVAs was 40-150 μm after 2005 and 100-500 μm before then. A protocol computed tomography (CT) scan was performed 1 month after TAE, and subsequent treatments were decided upon according to residual vascularity. A subset of patients was randomized to TACE between 2003 and 2010 as part of a clinical trial.[16] Eighteen patients who eventually underwent transplantation were randomized, 9 being allocated in the TACE (with cisplatin) group and 9 in the TAE group. In the trial no significant difference in survival between the 2 therapies was demonstrated.[16] No patient had TAE/TACE in the month before LT.

All patients had protocol imaging studies [CT or magnetic resonance imaging (MRI)] while on the transplant waiting list every 3 months until LT, for the evaluation of potential HCC progression. Chest and liver CT were performed as a standard protocol in all patients after LT every 6 months in order to diagnose tumor recurrence. All patients undergoing transplantation were followed up at least once every 6 months, until death or completion of study.

Histopathologic evidence of tumor necrosis after transarterial therapy was evaluated in the explanted livers and classified as follows: (1) complete necrosis, in which only necrotic material and no viable cancer cells were visible; (2) partial necrosis, in which both necrotic material and residual tumor were identified; and (3) no necrosis, in which tumor was identified with no evidence of necrotic material. All tumor lesions were measured and compared with the latest pre-LT radiological findings. Any discrepancy between the two was recorded.

Laboratory variables, including full blood count, alanine aminotransferase, aspartate aminotransferase, bilirubin, alkaline phosphatase, albumin, prothrombin time, and AFP were collected on the day of transplantation.

Inflammation-based Scores

NLR, PLR, and IBI were the inflammation-based scores evaluated in the study. NLR is the calculated ratio of blood neutrophil to lymphocyte count. PLR is calculated by dividing the absolute platelet count by the absolute lymphocyte count. IBI is a score that combines C-reactive protein (CRP) and serum albumin. Patients with a CRP $\geq$10 mg/dL and hypoalbuminemia (serum albumin was $<$ 35 g/L) are allocated a score of 2; those with either one of the abovementioned abnormalities have a score of 1, whereas patients with normal values (CRP was $<$10 mg/dL, albumin was $\geq$35 g/L) are allocated a score of 0.

These scores were calculated individually for every patient by laboratory values collected at 3 different time points, the day of their elective admission for LT and 1 month and 6 months after LT. There was no active infection (including positive bacterial cultures, antibiotic treatment for clinical suspicion of infection, or HIV positivity) in any of the patients who were included in the study.

Statistical Analysis

All data were analyzed in the statistical package SPSS (version 22.0; IBM, New York, NY). Comparisons of continuous variables between or among groups were made by *t* test and analysis of variance when quantitative variables were normally distributed. Distribution of nonparametric continuous variables in different groups was analyzed by Mann-Whitney or Kruskal-Wallis test. Qualitative data were compared

TABLE 1. Patient Characteristics

Number of patients	150
Median follow-up (months)	28 (range = 0-116)
Mean age (years)	54 ± 7
Etiology of liver disease, n (%)	
Hepatitis C virus	60 (40)
Hepatitis B virus	34 (23)
Alcohol	20 (13)
Unknown	36 (24)
Sex male, n (%)	125 (83)
Mean Child-Pugh score	7 ± 2
Median AFP (ng/mL)	13 (range = 2-67,380)
AFP >200 ng/mL, n (%)	13 (9)
TACE/TAE, n (%)	71 (47)
Median time on waiting list (months)	1.6 (range = 0-12)
HCC recurrence yes, n (%)	19 (13)
Median time to recurrence (months)	29 (range = 4-63)
Median NLR pre-LT	2.2 (range =1-14)
Median NLR at month 1 post-LT	4.8 (range = 1-43)
Median NLR at month 6 post-LT	2.5 (range = 0.5-29)
NLR ≥5 pre-LT, n (%)	19 (13)
NLR ≥5 at month 1 post-LT, n (%)	63/128 (49)
NLR ≥5 at month 6 post-LT, n (%)	10/91 (11)
Median PLR pre-LT	68 (range = 10-681)
Median PLR at month 1 post-LT	213 (range = 32-1120)
Median PLR at month 6 post-LT	135 (range = 25-1280)
PLR ≥150 pre-LT, n (%)	17 (11)
PLR ≥150 at month 1 post-LT, n (%)	89/128 (69)
PLR ≥150 at month 6 post-LT, n (%)	36/90 (40)
CRP (mg/dL)	6 (range = 1-80)
IBI score pre-LT, n (%)	0:35 (35.4); 1:46 (46); 2:18 (19)
IBI score at month 1 post-LT, n (%)	0:4 (4.5); 1:59 (65.5); 2:27 (30)
IBI score at month 6 post-LT, n (%)	0: 1(3); 1: 36 (78); 2: 9 (19)

NOTE: Continuous variables are expressed as mean ± standard deviation when distribution is normal. Nonparametric continuous variables are expressed as median and range. Categorical variables are described as n (%).

with corrected chi square or 2-tailed Fisher's exact test. Multivariate analysis used Cox regression. $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Between 1996 and 2010, 150 consecutive patients with HCC underwent transplantation and were included in the study. The predominant causes of chronic liver disease were chronic hepatitis C (40% of patients), chronic hepatitis B (23%) and alcohol abuse (13%). Mean age of the study population was 54 ± 7 years, and 125 (83%) were males. Mean Child-Pugh score was 7 ± 2. Seventy-one patients (47%) had transarterial embolization prior to LT. Thirteen patients (9%) had an AFP >200 ng/mL. Median time on the waiting list was 1.6 (range = 0-12) months, and median follow-up time was 28 (range = 0-116) months (Table 1).

Tumor Characteristics

Among the 150 patients included in the study, 107 (72%) fit the Milan criteria based on histopathological examination of the explanted liver. Thus, there was a 28% discrepancy between radiological and histological evaluation. In 19 patients, HCC was an incidental finding. Twelve patients (8%) had a poorly differentiated HCC, whereas microvascular invasion was found in 26%. Nineteen patients (13%) had an HCC recurrence. Median time to recurrence was 29 (range = 4-63) months. Two patients had a late HCC recurrence, one at 54 months and the other at 63 months post-transplantation. Because these tumors appeared in noncirrhotic livers, we classified them as recurrences rather than de novo tumors.

Complete necrosis was found in 22 of 71 (31%) explanted tumors in patients treated with TAE or TACE versus 54% with partial and 15% with no necrosis. All patients with complete necrosis did not have HCC recurrence at the completion of the follow-up.

Inflammation Characteristics

Median NLR of the study population was 2.2 (range = 1-14) on the day of liver transplantation, 4.8 (range = 1-43) 1 month post-LT, and 2.5 (range = 0.5-29) 6 months post-LT. Nineteen patients (13%) had an

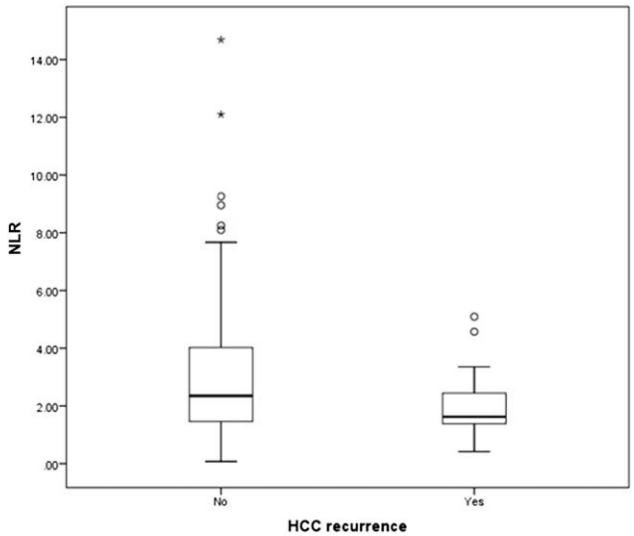

Figure 1. NLR was not significantly different between patients with and without HCC recurrence (median NLR 1.6 and 2.3, respectively; $P = 0.08$).

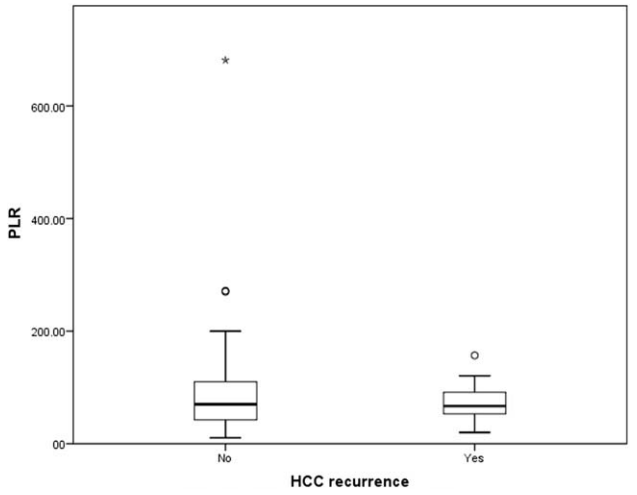

Figure 2. PLR was not significantly different between patients with and without HCC recurrence (median PLR 66 and 70, respectively; $P = 0.63$).

NLR ≥5 before LT, which has been used as a cutoff in other studies to predict tumor recurrence and worse outcome.[17]

Median PLR was 68 (range = 10-681) preoperatively. This was markedly increased on subsequent measurements, to 213 (range = 32-1120) at 1 month and 135 (range = 25-1280) at 6 months post-LT. PLR ≥150, which is a cutoff value previously associated with vascular invasion,[18] was found in 17 patients (11%) out of 128 the day of transplantation. Eighty-nine patients (69.5%) had a PLR ≥150 at 1 month after LT, and 36/90 (40%) patients had an elevated PLR 6 months after LT.

A normal IBI score was found in 35 out of 99 (35.4%) patients before LT; 46 patients had 1 abnormal parameter (scoring 1), and 18 patients out of 99 (19%) had both albumin and CRP above the normal range (IBI score of 2). Median CRP at the same time point was 6 (range = 1-80) mg/dL. Conversely, only 4 patients had a normal IBI score 1 month post-LT; 59 had a score of 1, and 30% had 2 abnormal parameters. Abnormality of IBI persisted 6 months post-LT; only 1% scored 0. Median CRP was 16.5 (range = 1-220) mg/dL and 4 (range = 1-189) at the 1st and 6th months post-LT, respectively (Table 1).

Inflammatory Scores as Predictors of HCC Recurrence and Survival

In our cohort of patients, NLR was not associated with post-LT HCC recurrence at any of the 3 different time points when it was measured ($P = 0.08$ before LT, $P = 0.74$ 1 month post-LT, $P = 0.09$ 6 months post-LT; Fig. 1). The correlation of an elevated NLR ≥5 with cancer relapse did not reach statistical significance ($P = 0.47$, $P = 0.42$, and $P = 1.00$ at LT and at 1 and 6 months after, respectively). Moreover, NLR was not significantly different between patients who had TAE/TACE and those who did not ($P = 0.1$). Distribution of PLR was not significantly different between patients with recurrence and those without ($P = 0.63$ at LT, $P = 0.24$ 1 month post-LT, $P = 0.15$ 6 months post-LT; Fig. 2). CRP, serum albumin, and calculated IBI score were not predictive of HCC recurrence on univariate analysis. In particular, no patient with the highest IBI score of 2 at LT experienced an HCC recurrence. Additionally, neither neutrophils nor lymphocytes were found to be significantly different between patients who experienced an HCC recurrence and those who did not (Table 2).

NLR was equally distributed in patients who underwent transplantation within or outside the Milan criteria ($P = 0.72$). Moreover, there was no significant association between total tumor size or number and elevated NLR ($P = 0.59$ and $P = 0.94$ respectively). Inflammation-based scores pre-LT were compared to response to transarterial neoadjuvant therapy as this was assessed by histology in the explanted liver. Median NLR was 1.5 (range = 0.7-4), 2.5 (range = 0.7-14), and 2.8 (range = 0.8-12) in patients with no, partial, and complete necrosis after TAE, respectively ($P = 0.03$; Fig. 3). When the analysis was repeated with a cutoff NLR value of 5, there was no significant difference ($P = 0.25$). Lymphocytes were not associated with the difference in NLR; they were distributed homogeneously between patients who responded to TAE and nonresponders ($P = 0.7$). There were significantly more neutrophils in patients who completely responded to TAE compared with those with partial and no response ($P = 0.03$).

The same pattern appeared in the analysis of PLR compared with no, partial, or complete tumor necrosis. Increased PLR was significantly associated with complete tumor necrosis after TAE on univariate analysis ($P = 0.02$). Platelets were significantly increased in TAE responders compared with partial responders

TABLE 2. Univariate Analysis of Inflammation Scores as Predictors of HCC Recurrence Based on 150 Patients

	HCC Recurrence	No Recurrence	*P* Value
Patients, n (%)	19 (13)	131 (87)	
Median NLR pre-LT	1.6 (range = 0.4-5)	2.3 (range = 1-14)	0.08
NLR ≥5 pre-LT, n (%)	1 (5)	18 (13.7)	0.47
Median PLR pre-LT	66 (range = 20-156)	70 (range = 10-681)	0.63
PLR ≥150 pre-LT, n (%)	1 (5)	16 (12)	0.47
Median CRP (mg/dL)	3 (1-80)	7 (range 1-77)	0.18
Median neutrophils count ($\times 10^3$/mL)	1.9 (range = 0.7-6.4)	2.6 (range = 0.5-8)	0.1
Median lymphocytes count ($\times 10^3$/mL)	0.9 (range = 0.4-3.1)	1.1 (range= 0.1-6.9)	0.74
IBI score, n (%)	0:4 (33); 1:8 (66)	0:31 (35); 1:38 (43); 2:18 (20)	0.16
Median NLR at month 1 post-LT	6 (range = 2.5-14.3)	4.7 (range = 1-43)	0.74
Median NLR at month 6 post-LT	3 (range =1.8-6.4)	2.5 (range = 0.5-29)	0.09
Median PLR at month1 post-LT	279 (range = 89-594)	211 (range = 32-1120)	0.24
Median PLR at month 6 post-LT	155 (range = 120-286)	130 (range = 25-1280)	0.15

NOTE: Nonparametric continuous variables are expressed as median (range). Categorical variables are expressed as n (%).

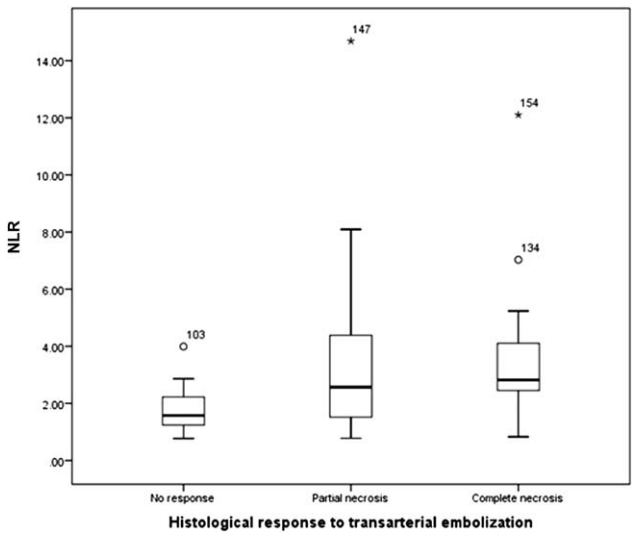

Figure 3. NLR was significantly higher in patients with complete necrosis post-TACE/TAE versus those with partial or no necrosis (median NLR 2.8, 2.5, and 1.5 respectively; $P = 0.03$).

or nonresponders ($P = 0.03$). IBI was not associated with response to TAE, nor were CRP and serum albumin ($P = 0.72$, $P = 0.33$, and $P = 0.79$, respectively). Moreover, NLR and PLR at 1 and 6 months post-LT were not associated with tumor necrosis in patients treated preoperatively with TAE (data shown in Table 2).

Inflammatory scores were also analyzed to detect any association with overall survival of our cohort, but no score was significantly associated with survival. Preoperative NLR ≥5 did not predict worse survival ($P = 0.88$), nor did PLR ≥150 ($P = 0.64$) or high IBI ($P = 0.15$). To avoid any selection bias, statistical analysis was repeated after exclusion of patients who died within 1 month of LT (n = 6), and results did not substantially change (data not shown).

Predictors of HCC Recurrence

On univariate analysis, post-LT HCC recurrence was associated with tumor outside the Milan criteria histologically ($P = 0.001$), summation of total diameter either histologically or radiologically of tumor nodules ($P = 0.002$ and $P = 0.009$ respectively), diameter of the largest nodule either radiologically or histologically ($P = 0.001$ both), no transarterial embolization ($P = 0.009$), absence of histological response to TAE ($P = 0.002$), microvascular and macrovascular invasion ($P = 0.048$ and $P = 0.046$, respectively), and pre-LT value of AFP >200 ng/mL ($P = 0.003$). Tumor grade and presence of multifocal or satellite nodules were not associated with HCC recurrence ($P = 0.13$, $P = 0.62$, and $P = 0.2$, respectively).

To correct for the different policies regarding TAE over time, the period before and after 2001 was entered in the multivariate analysis as a binary variable. On Cox regression analysis, absence of neoadjuvant transarterial therapy (OR = 4.33, 95% CI = 1.28-14.64; $P = 0.02$) and no fulfillment of the Milan criteria in the explanted liver (OR = 3.34, 95% CI = 1.08-10.35; $P = 0.04$) were the independent predictors of post-LT HCC recurrence. Results did not substantially change when the analysis was performed after exclusion of incidental HCCs in the explanted liver or after the exclusion of patients who died within 1 month from LT (n = 6).

DISCUSSION

The main finding of our study was that inflammation-based scores were not associated with HCC recurrence after LT. This finding is in contrast to the majority of similar previously published studies, which have associated such scores with HCC relapse and/or worse overall survival.[11,12,17,19,20]

To date, inflammatory markers and particularly inflammation-based scores have been described as

predictors of cancer-related prognosis and survival in several malignancies, such as colorectal, breast, lung, and gastric cancers.[14,21,22] The theoretical background for the possible predictive value of these markers lies in the close association of chronic inflammation and carcinogenesis. Host responses via innate and adaptive immune systems may result in a worse prognosis of HCC. CD8 T cells and natural killer cells are protective against tumor proliferation, and these are decreased in HCC.[23] In contrast, increased intratumor $CD66b^+$ neutrophils have been identified independently with poor HCC outcome.[24]

NLR has been analyzed most often quantitatively, but also several cutoff values have been described as predictors of worse cancer prognosis. Predominantly, a cutoff NLR $\geq$5 was associated with worse HCC outcome and survival.[17] NLR in HCC was first evaluated by Gomez et al.,[12] who showed that a preoperative cutoff value of $\geq$5 was independently associated with poor disease-free survival after hepatic resection. Halazun et al.[20] expanded this further, showing that increased preoperative NLR was associated with post-LT HCC recurrence and recipient death. This finding was noted in other studies[7,18,19,25] in which an elevated pre-LT NLR was independently associated with cancer relapse and poor overall survival post-LT. Moreover, in 2 studies, patients with intermediate- or advanced-stage HCCs who were embolized[26] or treated with sorafenib[27] had disease progression and overall survival that correlated with their baseline NLRs.

PLR is a less well-studied inflammatory marker that has been described as a predictor of post-LT tumor recurrence by Lai et al.[18] IBI is a novel inflammatory score that has been validated as being associated independently with overall survival in an independent cohort of 466 HCC patients.[15] The GPS and modified GPS also include CRP and serum albumin and have both been associated with poor prognosis in different HCC stages.[11]

There are potential explanations for why we found no association of inflammatory markers with HCC recurrence or survival after LT compared with the previously published literature. As shown in Table 3, the mean NLR in our cohort was significantly less than that in other studies; 12.7% had an NLR >5 compared with 20% in studies by Yoshizumi et al. and Lai et al. All patients in our cohort were within the Milan criteria, and the discrepancy between radiological and histopathological staging was 28%. In previous studies, fewer patients underwent transplantation within Milan criteria, and the discrepancy between radiological and histopathological evaluation was up to 34%.[19] Tumor characteristics in our population were also more favorable; poor differentiation and microvascular invasion were found in 8% and 26%, respectively. In addition, we included only patients who eventually underwent LT, whereas in the study by Lai et al.[18] transplant waiting list dropouts were also evaluated. Therefore, our population of HCC patients had more favorable tumor characteristics overall compared with those in similar studies (Table 3). However, it should be noted that NLR was not associated with number or size of HCC lesions. Our time to recurrence after LT (32 months) was longer than in other studies despite time on waiting list being significantly shorter.[7,18]

NLR, PLR, and IBI are ratios that consist of parameters that can easily be affected by infection, chronic disease, and other similar factors. Cirrhosis is a condition in which cellular immune response is compromised. T-lymphocyte subpopulations are deficient in cirrhosis, and this deficiency is further worsened when chronic liver disease is complicated by infection.[28] In addition, bacterial translocation and circulating endotoxin contribute to a proinflammatory state. Thus, a decreased lymphocyte count or an infection-induced high neutrophil count may frequently be found in chronic liver disease independently of the presence of cancer. CRP, a component of the IBI score, is a generic marker that can be elevated in several inflammatory conditions. Serum albumin may be affected by nutritional status and is associated with severity of liver disease, regardless of HCC. Thus, inflammation-based scores and their alterations could represent several other aspects of disease that are not necessarily associated with cancer. This conclusion is further confirmed by the fact that, even though NLR, PLR, and IBI were substantially increased when measured after LT, they still were not associated with HCC outcome, possibly representing postsurgical or immunosuppression-related sequelae.

Moreover, inflammatory ratios are calculated from peripheral blood tests and are far from reflecting the tumor microenvironment itself. Increased intratumoral neutrophils and CD8 T-lymphocyte depletion are found in HCC tissues and are associated with HCC progression, but these immune-driven alterations are not reflected directly in peripheral blood.[29]

In our study, patients who fully responded to neoadjuvant transarterial embolization with no viable cancer in their explanted HCCs had increased NLR and PLR ratios before LT compared with those who had a partial or no response to TAE. This elevation was due predominantly to a significantly higher neutrophil and platelet count in TAE responders versus nonresponders, because lymphocytes were equally distributed in both populations. HCC embolization results in activation of the host immune system and release of tumor-specific antigens and proinflammatory cytokines.[30] Adequate activation of the host immune response to tumor may lead to elevation of peripheral inflammatory cells, which could be more profound in those patients who responded to therapy and achieved an HCC necrosis. In a study by Huang et al.,[30] patients who had increased NLR post-TAE had better outcomes, which may reflect the level of tumor necrosis. Confirmation of this association in further studies is needed to recommend safely the use of inflammatory scores to indicate response to TAE and even guide the decision for repeat TAE sessions.

The only independent predictors of HCC recurrence in our study were absence of fulfillment of the Milan

TABLE 3. Comparative Characteristics of Studies Assessing Post-LT HCC Recurrence

	Halazun et al.,[20] (2009)	Bertuzzo et al., [19] (2011) T	Yoshizumi et al., [7] (2013)	Lai et al.,[18] (2014)	Current study
Number of patients	150	219	167	146 transplanted	150
Median follow-up (months)	36	40	57	N/A	28
Mean age (years)	57	57	54	58	54
Main etiology of liver disease (%)	HCV 69	HCV 34	HCV 46	HCV 43	HCV 40
Sex, male, (%)	79	84	60	80	83
Median MELD	N/A	16	11.6	11	N/A
Mean CTP	N/A	N/A	N/A	N/A	7 ± 2
Median AFP (ng/mL)	N/A	10 (cutoff above 30 ng/mL 30%)	1516 (cutoff above 400 ng/mL 20%)	8.5 (cutoff above 200 ng/mL 7%)	13 (2-67,380) (cutoff above 200 ng/mL 10%)
Median NLR	N/A	3.15	3.1	2.9	2.2
NLR >5 (%)	9	14	>4: 20	>5.4: 20	12.7
Median PLR	N/A	N/A	N/A	92	68
PLR >150 (%)	N/A	N/A	N/A	28	11
TAE/TACE yes (%)	89	51	56	71	47
Median time to LT (months)	N/A	N/A	40	N/A	1.6
Radiological Milan criteria yes, (%)	70	74	50	79	100
Histological Milan criteria, yes, (%)	N/A	65	N/A	N/A	72
Poor tumor differentiation, yes (%)	N/A	32	32	N/A	8
Microvascular invasion, yes (%)	N/A	57	37	37	26
Macrovascular invasion, yes (%)	N/A	1.4	N/A	N/A	4.7
Portal vein thrombosis, (%)	N/A	1.5	N/A	N/A	N/A
HCC recurrence, yes (%)	19	17.9	18	9.6	13
Median time to recurrence (months)	N/A	N/A	N/A	20	29
Multivariate analysis: predictors of HCC recurrence	Kaplan-Meier: NLR, tumor size	Cox regression: MVI, NLR >5	Cox regression: total tumor size, HCC number >8, NLR >4	Kaplan-Meier: AFP >200, PLR >150	Cox regression: no transarterial therapy, out of Milan criteria histologically
Median preoperative tumor number. n	N/A	2	N/A	N/A	1
Preoperative largest tumor diameter (cm)	N/A	2.6	2.4	2.5	2.7
Tumor number upon histologic analysis, n	N/A	2	N/A	N/A	1
Largest tumor diameter upon histologic analysis (cm)	N/A	2.3	N/A	N/A	2.5
Partial/total necrosis post TAE (%/%)	N/A	40/20	N/A	N/A	54/31
Down-staging and enlistment, (%)	N/A	15	N/A	N/A	N/A

criteria in the explanted liver and the absence of TAE treatment, factors described previously.[9] Tumor size appears to be the most reproducible predictor of HCC recurrence post-LT in numerous studies. Molecular research in intratumoral and peritumoral tissue could potentially identify novel markers that predict HCC recurrence. However, the need for biopsy from tumoral and nontumoral areas is the major limitation of such methods.[31]

In conclusion, we failed to demonstrate a significant association between the inflammatory scores NLR, PLR, and IBI at various time points and HCC recurrence post-LT in patients within the Milan criteria. Subsequent studies, mostly at the molecular level, could better clarify the immune alterations taking place in HCC pre- and posttransplantation and reveal more specific and accurate serum inflammatory predictors of HCC recurrence.

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REFERENCES

1. Bosch FX, Ribes J, Cleries R, Diaz M. Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2005;9:191-211.
2. Tsochatzis EA, Germani G, Burroughs AK. Transarterial chemoembolization, transarterial chemotherapy, and intra-arterial chemotherapy for hepatocellular carcinoma treatment. Semin Oncol 2010;37:89-93.
3. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693-699.
4. Germani G, Theocharidou E, Adam R, Karam V, Wendon J, O'Grady J, et al. Liver transplantation for acute liver failure in Europe: outcomes over 20 years from the ELTR database. J Hepatol 2012;57:288-296.
5. Zimmerman MA, Ghobrial RM, Tong MJ, Hiatt JR, Cameron AM, Hong J, et al. Recurrence of hepatocellular carcinoma following liver transplantation: a review of preoperative and postoperative prognostic indicators. Arch Surg 2008;143:182-188.
6. Duvoux C, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al.; for Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including alpha-fetoprotein improves the performance of Milan criteria. Gastroenterology 2012;143:986-994 e3; quiz e14-e15.
7. Yoshizumi T, Ikegami T, Yoshiya S, Motomura T, Mano Y, Muto J, et al. Impact of tumor size, number of tumors and neutrophil-to-lymphocyte ratio in liver transplantation for recurrent hepatocellular carcinoma. Hepatol Res 2013;43:709-716.
8. Rodríguez-Perálvarez M, Tsochatzis E, Naveas MC, Pieri G, García-Caparrós C, O'Beirne J, et al. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. J Hepatol 2013;59:1193-1199.
9. Tsochatzis E, Garcovich M, Marelli L, Papastergiou V, Fatourou E, Rodriguez-Peralvarez ML, et al. Transarterial embolization as neo-adjuvant therapy pretransplantation in patients with hepatocellular carcinoma. Liver Int 2013;33:944-949.
10. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539-545.
11. Kinoshita A, Onoda H, Imai N, Iwaku A, Oishi M, Tanaka K, et al. The Glasgow prognostic score, an inflammation based prognostic score, predicts survival in patients with hepatocellular carcinoma. BMC Cancer 2013;13:52.
12. Gomez D, Farid S, Malik HZ, Young AL, Toogood GJ, Lodge JP, et al. Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. World J Surg 2008;32:1757-1762.
13. Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O'Reilly DS, et al. A comparison of inflammation-based prognostic scores in patients with cancer. A Glasgow Inflammation Outcome Study. Eur J Cancer 2011;47:2633-2641.
14. Malietzis G, Giacometti M, Askari A, Nachiappan S, Kennedy RH, Faiz OD, et al. A preoperative neutrophil to lymphocyte ratio of 3 predicts disease-free survival after curative elective colorectal cancer surgery. Ann Surg 2014;260:287-292.
15. Pinato DJ, Stebbing J, Ishizuka M, Khan SA, Wasan HS, North BV, et al. A novel and validated prognostic index in hepatocellular carcinoma: the inflammation based index (IBI). J Hepatol 2012;57:1013-1020.
16. Meyer T, Kirkwood A, Roughton M, Beare S, Tsochatzis E, Yu D, et al. A randomised phase II/III trial of 3-weekly cisplatin-based sequential transarterial chemoembolisation vs embolisation alone for hepatocellular carcinoma. Br J Cancer 2013;108:1252-1259.
17. Oh BS, Jang JW, Kwon JH, You CR, Chung KW, Kay CS, et al. Prognostic value of C-reactive protein and neutrophil-to-lymphocyte ratio in patients with hepatocellular carcinoma. BMC Cancer 2013;13:78.
18. 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. Transpl Int 2014;27:32-41.
19. Bertuzzo VR, Cescon M, Ravaioli M, Grazi GL, Ercolani G, Del Gaudio M, et al. Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. Transplantation 2011;91:1279-1285.
20. Halazun KJ, Hardy MA, Rana AA, Woodland DC 4th, Luyten EJ, Mahadev S, et al. Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. Ann Surg 2009;250:141-151.
21. Lee DY, Hong SW, Chang YG, Lee WY, Lee B. Clinical significance of preoperative inflammatory parameters in gastric cancer patients. J Gastric Cancer 2013;13:111-116.
22. Tomita M, Shimizu T, Ayabe T, Nakamura K, Onitsuka T. Elevated preoperative inflammatory markers based on neutrophil-to-lymphocyte ratio and C-reactive protein predict poor survival in resected nonsmall cell lung cancer. Anticancer Res 2012;32:3535-3538.
23. Chen L, Zhang Q, Chang W, Du Y, Zhang H, Cao G. Viral and host inflammation-related factors that can predict the prognosis of hepatocellular carcinoma. Eur J Cancer 2012;48:1977-1987.
24. Li YW, Qiu SJ, Fan J, Zhou J, Gao Q, Xiao YS, et al. Intratumoral neutrophils: a poor prognostic factor for hepatocellular carcinoma following resection. J Hepatol 2011;54:497-505.
25. Motomura T, Shirabe K, Mano Y, Muto J, Toshima T, Umemoto Y, et al. Neutrophil-lymphocyte ratio reflects

hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. J Hepatol 2013;58:58-64.

26. McNally ME, Martinez A, Khabiri H, Guy G, Michaels AJ, Hanje J, et al. Inflammatory markers are associated with outcome in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization. Ann Surg Oncol 2013;20:923-928.
27. Zheng YB, Zhao W, Liu B, Lu LG, He X, Huang JW, et al. The blood neutrophil-to-lymphocyte ratio predicts survival in patients with advanced hepatocellular carcinoma receiving sorafenib. Asian Pac J Cancer Prev 2013;14: 5527-5531.
28. Lagadinou M, Solomou EE, Velissaris D, Theodorou GL, Karakatza M, Gogos CA. Alterations in T-lymphocyte subpopulations in patients with complicated liver cirrhosis. Diagn Microbiol Infect Dis 2013;75:348-356.
29. Huang Y, Wang FM, Wang T, Wang YJ, Zhu ZY, Gao YT, et al. Tumor-infiltrating FoxP3+ Tregs and $CD8^+$ T cells affect the prognosis of hepatocellular carcinoma patients. Digestion 2012;86:329-337.
30. Huang ZL, Luo J, Chen MS, Li JQ, Shi M. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization. J Vasc Intervent Radiol 2011;22:702-709.
31. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 2008;359:1995-2004.