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ORIGINAL ARTICLE

Neutrophil and platelet-to-lymphocyte ratio as new predictors of dropout and recurrence after liver transplantation for hepatocellular cancer

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Conflict of interest
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Summary

There is increasing evidence that systemic inflammation markers like neutrophil (NLR) and platelet-to-lymphocyte ratios (PLR) may play a role in the outcome of hepatocellular cancer (HCC). Between January 1994 and March 2012, 181 patients with HCC were registered on the transplant waiting list: 35 (19.3%) patients dropped out during the waiting period and 146 (80.7%) patients underwent liver transplantation (LT). The median follow-up of this patient cohort was 4.2 years (IQR: 1.8–8.3). On c-statistics, the last NLR (AUROC = 67.4; $P = 0.05$) was the best predictor of dropout. The last PLR had an intermediate statistical ability (AUROC = 66.1; $P = 0.07$) to predict post-LT tumor recurrence. Patients with a NLR value >5.4 had poor 5-year intention-to-treat (ITT) survival rates (48.2 vs. 64.5%; $P = 0.02$). Conversely, PLR better stratified patients in relation to tumor-free survival (TFS) (80.7 vs. 91.6%; $P = 0.02$). NLR is a good predictor for the risk of dropout, while PLR is a good predictor for the risk of post-LT recurrence. Use of these markers, which are all available before LT, may represent an additional tool to refine the selection criteria of HCC liver recipients.

Introduction

Hepatocellular cancer (HCC) is the fifth cause of tumor-related death worldwide [1]. Liver transplantation (LT) is a curative treatment option for well-selected cirrhotic patients; moreover, this treatment has the advantage to cure not only the cancer but also the underlying carcinogenic liver disease [2]. The Milan criteria (MC), introduced in clinical practice in 1996 to optimize the oncologic results of LT, have been proven to be too restrictive. Several groups therefore have widened these criteria, mostly by expanding tumor diameter and number [3–7]. The extension of the inclusion criteria, however, made it rapidly clear that they needed to be combined with parameters that take into account biological behavior and aggressiveness of the tumor in order to obtain similar outcomes after LT. Other

surrogate markers of microvascular invasion (mVi), a finding associated with an increased risk of tumor recurrence, have therefore been sought [6,8–11]. Recently, systemic inflammation has been linked to poor outcome and increased tumor progression. This is due to the fact that the tumor up-regulates the inflammatory process which in turn predisposes to tumor proliferation and development of metastases through inhibition of apoptosis, promotion of angiogenesis, and DNA damage [12–14]. Such systemic inflammatory responses have been investigated using markers such as the elevation of the neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte ratios (PLR). Inflammatory markers have been shown to be prognostic parameters in other gastroenterological malignancies as well as in HCC [15–24]. Elevated markers have been shown to significantly increase the risk for recurrence after different kinds of treatment for HCC such as liver resection, transarterial chemo-embolization (TACE), radio-frequency destruction (RF) and even LT [18–24]. Several studies have already focused on the impact of these parameters on recurrence after LT, but so far none have investigated their role as possible selection tools of potential liver recipients or as factors predicting dropout on the waiting list.

The aim of this study was to evaluate NLR and PLR as potential risk factors for dropout before and for recurrence after LT. Both markers were also compared to alpha-fetoprotein (AFP), a known prognostic factor for HCC, in order to better understand their impact on dropout before and recurrence after LT.

Materials and methods

Data collection

Between January 1994 and March 2012, 211 patients with a pre-LT proven diagnosis of HCC entered the waiting list (WL) for LT at our institution. Twenty-six patients with a follow-up less than 24 months and four patients lacking data were excluded from the analysis. Thirty-five (19.3%) of 181 patients dropped out during the waiting time; 146 (80.7%) patients were transplanted (Fig. 1). The characteristics of the overall, dropped out, and transplanted patient cohorts are displayed in Table 1.

Diagnosis and HCC staging

Diagnosis of HCC was made if typical features were present on two different imaging modalities such as ultrasound, computed tomography (CT), or magnetic resonance (MR) scan and/or one imaging modality supported by AFP level >400 ng/ml [25]. If imaging was inconclusive, the diagnosis was confirmed by image-guided biopsy. All the imaging procedures were performed and reviewed by the same team of expert radiologists working in our hospital.

From 1996 onwards, the MC were adopted as selection criteria for registration on the WL; patients exceeding these criteria while waiting were removed from the list. From 2001 onwards, inclusion criteria were extended to the University of California San Francisco criteria (UCSFC). These criteria were considered the acceptable upper limit of tumor progression during the waiting time. All patients exceeding UCSFC during this period dropped out from the waiting list.

Treatment of HCC on the LT wait list

Locoregional treatment (LRT) was performed in accordance with the pre-LT guidelines proposed by the European Association for the Study of the Liver (EASL) [25]. LRT consisted of TACE, RF, percutaneous ethanol injection (PEI), and partial liver resection (LR).The number of sessions was determined by clinical and biochemical changes during LRT.

Assessment of radiological and biological response during the waiting time

Patients were followed-up monthly after LRT by CT or MR scan. Radiological response after LRT was evaluated according to the mRECIST (modified Response Evaluation Criteria in Solid Tumours) criteria [26]. Progressive disease was defined as an increase of at least 20% of the sum of the largest diameters of the target lesion (with an absolute increase of at least 5 mm) or the appearance of new lesions.

Assessment of response to pre-LT treatments using mRECIST was retrospectively performed by our radiologists in all the cases transplanted before mRECIST introduction in the clinical practice.

Alpha-fetoprotein, NLR, and PLR values were taken at two well-defined time points, at registration on the waiting list and at moment of dropout or LT. Slopes of progression were calculated according to Vibert, considering the difference between initial and final values divided by the number of months (time) between the two referral points [27].

Dropout and post-LT patient follow-up

Dropout from the WL was defined as exclusion from the list or patient death, irrespective if directly or indirectly related to HCC. Tumor-related causes of dropout were progression beyond conventional criteria of transplantability (i.e., macrovascular invasion or extrahepatic metastases) or patient death due to liver failure after TACE for the treatment of a progressing HCC.

All transplanted patients were followed-up in the same LT outpatient clinic. Screening for tumor recurrence was carried out by repetitive AFP measurement and by 3-monthly Doppler ultrasound and abdominal scanning. A routine thoraco-abdominal CT scan was

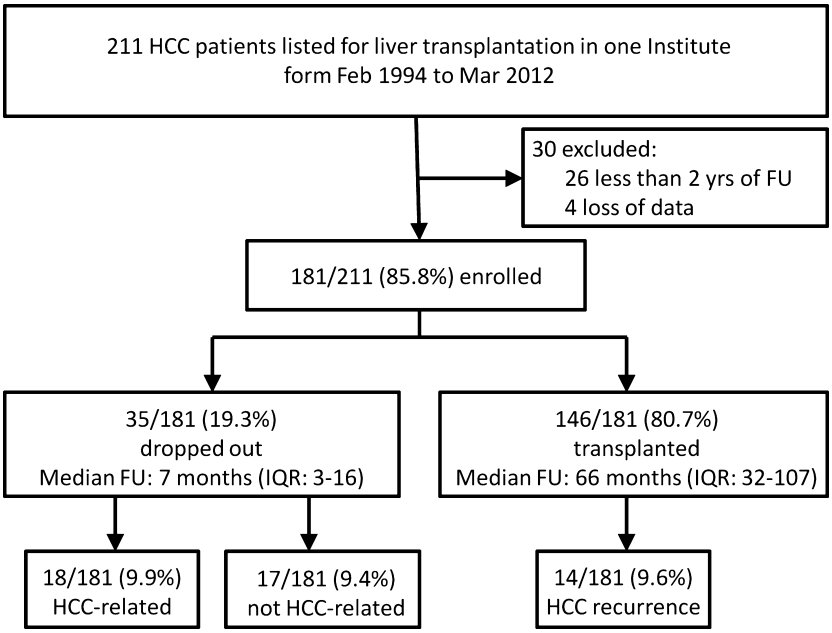

Figure 1 Flow chart of patients excluded and included in the study.

performed half yearly or yearly depending on the outcome of the histological examination. Bone scan was also carried out in case of suspected HCC recurrence. No patient received adjuvant chemotherapy. As of October 31, 2012, the median follow-up of the entire population from moment of WL registration was 4.2 years (interquartile ranges [IQR]: 1.8–8.3).

Statistical analysis

Categorical variables were reported as the number of cases and percentages; continuous variables were given as median (IQR) values.

Different AFP, NLR and PLR values, namely (i) at the moment of waiting list registration, (ii) at the moment of LT or dropout, and (iii) their slopes of progression during the waiting list period, were tested using c-statistics analysis, with the intention to analyze their ability to predict dropout and post-LT recurrence. The best predictors for each parameter were established according to the higher area under the receiver operating characteristic (AUROC) curve. Cutoff values of these variables were investigated. The diagnostic odds ratio (DOR), which measures the overall accuracy of a diagnostic test, was calculated for each cut-off value. A higher DOR indicates a higher accuracy of the test [28]. Previously reported threshold values of AFP, AFP slope, NLR, and PLR were adopted [17,27,29]; third quartile values, respectively, corresponding to 0.1 and 2.9/month, were used for NLR and PLR slopes.

Intention-to-treat (ITT) survival and tumor-free survival (TFS) were investigated using the Kaplan–Meier method. ITT survival was defined as the time interval between date of WL registration and of death or dropout (from any cause). TFS was defined as the time interval between date of LT and of HCC recurrence. Investigated variables included waiting time, Model for End-Stage Liver Disease (MELD), diameter of the main lesion, number of tumors, MC and UCSFC status, pre-LT tumor treatment (yes vs. no), period of LT (before or after 2001), initial and final AFP, NLR and PLR values, and their respective slopes. Statistical significance was reached at $P < 0.05$. Statistical analyses and plots were performed with SPSS 19.0 (SPSS, Chicago, IL, USA).

Results

Dropout and recurrence rate

During the waiting time, 18 (9.9%) on 181 patients dropped out or died on the WL from HCC-related causes: 14 patients presented with HCC progression exceeding the selection criteria and four had a tumor-related death. In addition, 17 (9.4%) patients dropped out or died due causes unrelated to HCC: eight patients died from terminal liver failure and nine patients were excluded from the waiting list due to worsening of their status (6 patients) and poor compliance (3 patients).

One hundred forty-six (80.7%) patients were transplanted. Fourteen (9.6%) patients developed recurrence at a median post-LT period of 20.2 months (IQR 5.3–26.7); five (35.7%) patients recurred within the first post-LT year.

Table 1. Characteristics of the overall cohort, dropped out, and transplanted patient cohorts regarding recipient, tumor and pretransplant work-up. Percentages are calculated according to the number of cases reported in each column.

Variables	Patients listed for LT (n = 181) Median (IQR) or n (%)	Patients dropped out (n = 35) Median (IQR) or n (%)	Patients transplanted (n = 146) Median (IQR) or n (%)
Recipient			
Male gender	140 (77.3)	24 (68.6)	116 (79.5)
Age at list inscription (yrs)	58.3 (53.8–63.2)	59.8 (54.4–64.2)	58.0 (53.5–63.0)
Waiting time (months)	6.0 (2.6–10.0)	6.0 (2.0–11.0)	7.8 (2.7–10.0)
>6 months	82 (45.3)	16 (45.7)	66 (45.2)
LabMELD*	11 (8–14)	15 (6–19)	11 (8–11)
>15	43 (23.8)	12 (34.3)	31 (21.2)
HCV	74 (40.9)	11 (31.4)	63 (43.2)
HBV	33 (18.2)	7 (20.0)	26 (17.8)
CTP score C	25 (13.8)	5 (14.3)	20 (13.7)
Donor			
Male gender	–	–	78 (53.4)
Age (yrs)	–	–	49.8 (33.3–66.7)
>60 years			48 (28.1)
Tumor			
Major lesion diameter (cm)†	2.5 (1.7–3.5)	2.6 (2.0–3.7)	2.5 (1.7–3.5)
>5 cm†	16 (8.8)	4 (11.4)	12 (8.2)
Number of lesions†	1 (1–2)	1 (1–2)	1 (1–2)
>3†	13 (7.2)	4 (11.4)	9 (6.2)
MC-out†	41 (22.7)	9 (25.7)	32 (21.9)
UCSFC-out†	29 (16.0)	7 (20.0)	22 (15.1)
Pretransplant work-up			
LRT‡	168 (92.8)	32 (91.4)	136 (93.2)
TACE	134 (74.0)	30 (85.7)	104 (71.2)
PEI	60 (33.1)	10 (28.6)	50 (34.2)
RF	18 (9.9)	2 (5.7)	16 (11.0)
LR	12 (6.6)	3 (8.6)	9 (6.2)
Total number LRT	3 (1–4)	2 (2–5)	3 (1–4)
mRECIST progression§	32 (19.0)	16 (50.0)	16 (11.8)
Dropout	35 (19.3)	35 (100)	–

AFP, alpha-fetoprotein; CTP, Child-Pugh-Turcotte; HBV, hepatitis B virus; HCV, hepatitis C virus; LR, liver resection; LRT, loco-regional therapy; LT, liver transplantation; MC, Milan criteria; MELD, model for end-stage liver disease; mRECIST, modified Response Evaluation Criteria In Solid Tumours; NLR, neutrophil-to-lymphocyte ratio; PEI, percutaneous ethanol injection; PLR, platelet-to-lymphocyte ratio; RF, radio frequency; TACE, transarterial chemo-embolization; UCSFC, University of California san Francisco criteria.

*Lab-MELD calculation according to laboratory values.

†Radiological determination at the moment of waiting list inscription.

‡In 64 cases, two or more procedures were performed in the same patient.

§Percentage calculated only on the patients treated with LRT.

Biological markers as predictors of dropout and post-LT recurrence

Initial values of AFP, NLR, and PLR at the moment of waiting list registration, their final values before dropout or LT, and their dynamics during the waiting time are displayed in Table 2.

Interestingly, the final median values of NLR and PLR both increased when compared to their initial ones in the subgroup of dropped-out patients, while this behavior was not observed in the subgroup of transplanted patients.

On c-statistics, the last NLR was the best prognostic factor of dropout, with an AUROC curve of 67.4 (95% CI: 56.7–78.0; P = 0.05). NLR cut-off value of 5.4, corresponding to its third quartile, gave a DOR of 3.4. Last AFP and PLR values had intermediate predictive abilities.

When analyzing post-LT tumor recurrence, the last AFP value was the best prognostic test, with an AUROC curve of 70.6 (95% CI: 56.3–84.9; P = 0.02). The last PLR had an intermediate statistical ability (AUROC: 66.1; P = 0.07). NLR values showed a poor ability in predicting recurrence.

Intention to treat and tumor-free survival analyses

When analyzing ITT survival, variables related to tumor morphology (diameter and number of nodules, MC and

Table 2. NLR, PLR and AFP values at the moments of wait list inscription, LT or dropout and their dynamics during the waiting time.

Variables	Patients listed for LT (n = 181) Median (IQR) or n (%)	Patients dropped out (n = 35) Median (IQR) or n (%)	Patients transplanted (n = 146) Median (IQR) or n (%)
Initial AFP value (ng/ml)	9.6 (4.7 to 43.9)	19.5 (5.9 to 62.5)	8.5 (4.3 to 41.2)
>200 ng/ml	15 (8.3)	5 (14.3)	10 (6.8)
AFP before LT or DO (ng/ml)	9.1 (4.0 to 56.8)	14.0 (4.4 to 104.0)	8.6 (3.9 to 40.0)
>200 ng/ml	16 (8.8)	8 (22.9)	8 (5.5)
AFP slope (ng/ml/month)	−0.06 (−1.0 to 0.6)	−0.04 (−0.4 to 23.3)	−0.06 (−1.5 to 0.3)
Initial NLR value	2.9 (2.0 to 4.6)	2.9 (2.5 to 5.5)	2.9 (1.9 to 4.6)
>5.4	36 (19.9)	9 (25.7)	27 (18.5)
NLR before LT or DO	3.3 (1.8 to 5.4)	4.1 (3.0 to 10.3)	2.9 (1.7 to 5.0)
>5.4	45 (24.9)	15 (42.9)	30 (20.5)
NLR slope (month)	0 (−0.2 to 0.1)	−0.1 (−0.6 to 0)	0 (−0.1 to 0.2)
Initial PLR value	95.2 (69.8 to 135.6)	90.2 (63.1 to 141.7)	97.7 (70.7 to 135.4)
>150	36 (19.9)	6 (17.1)	30 (20.5)
PLR before LT or DO	95.1 (65.2 to 139.0)	112.0 (66.3 to 155.6)	92.3 (64.1 to 129.7)
>150	38 (21.0)	10 (28.6)	28 (19.2)
PLR slope (month)	0 (−4.9 to 2.9)	−0.1 (−16.0 to 0.3)	0 (−3.9 to 3.3)

AFP, alpha-fetoprotein; DO, dropout; LT, liver transplantation; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

UCSFC status) and to LT management (waiting time, LRT, MELD, era of LT) failed to demonstrate statistical significance. The last AFP value had the best stratification of patients in relation to ITT survival; 5-year survival rates in patients exceeding 200 ng/ml were 15.0% vs. 64.0% in patients with a value beneath this threshold ($P < 0.0001$). Similarly, the last NLR value of over 5.4 also enabled the patients to be stratified well: those exceeding this cut-off value had a 5-year survival rate of 48.2% vs. 64.5% in those meeting the threshold value ($P = 0.02$).

At TFS analysis, patients exceeding the last AFP cut-off value of 200 ng/ml had a 5-year survival rate of 40.0% vs. 91.7% in patients meeting the value ($P < 0.0001$). The last PLR value >150 had a good ability to stratify patients in relation to TFS (91.6 vs. 80.7%; $P = 0.02$). No NLR value had a good ability to stratify patients in relation to TFS (Table 3 and Fig. 2).

Subanalysis 1: the role of viruses

Starting with the consideration that not only the tumor, but also hepatic viruses might play a role in inducing inflammatory responses and immunoregulatory mechanisms that could affect tumor progression, a subanalysis was performed with the intention to evaluate the ITT survival in homogeneous subcohorts of patients having only positivity for HCV, HBV or no viral disease. In 69 (38.1%) patients, the only cause of cirrhosis was HCV infection, in 28 (15.5%) patients HBV infection; 79 (43.6) patients had no viral pathology and five (2.8%) patients with simultaneous HCV–HBV infection were excluded from this subanalysis. Interestingly, this subanalysis confirmed the results obtained when analyzing the entire patient cohort: the last NLR value of over 5.4 stratified patients well in the two subcohorts of HCV and nonviral patients. In contrast, this evidence was not observed in HBV-infected patients (Table 4).

Subanalysis 2: the role of LRT

This subanalysis aimed to evaluate the role of inflammatory markers in patients treated with LRT. Because of the small number of cases when considering all different types of locoregional treatment, we investigated only 134 patients treated with TACE. At ITT survival analysis, the last NLR value >5.4 stratified patients well (64.4 vs. 45.2%; in patients with NLR ≤ this value; $P = 0.04$), while last PLR >150 failed to do so (61.8 vs. 53.1; $P = 0.5$). In contrast, the last NLR was unable to stratify patients in relation to the risk of post-LT recurrence (90.5 vs. 86.9%; $P = 0.9$), while the last PLR >150 was able to do so (92.7 vs. 78.9; $P = 0.01$).

Discussion

Despite the fact that several markers have been used with the intent to guide prognosis in HCC, including morphological and biological parameters, none of them were of prognostic value. Recently, inflammatory markers have been considered for investigation of tumor progression and risk of HCC recurrence after LRT and LT. The importance of inflammatory markers as prognostic tools to predict outcome of different tumors has been underlined in a large study containing over 25 000 patients presenting with various malignancies [30]. Some studies related to HCC showed that the presence of an intratumoral inflammatory

Table 3. ITT survival and TFS of factors having an impact on dropout and post-LT recurrence.

	5-year ITT survival		5-year TFS	
Variables	%	*P*-value	%	*P*-value
Waiting time				
≤6 months	58.1	0.4	92.5	0.1
>6 months	61.1		85.7	
Lab-MELD				
≤15	75.5	0.2	91.8	0.1
>15	63.3		82.7	
Diameter major lesion				
≤5 cm	59.4	0.7	90.0	0.3
>5 cm	62.5		80.0	
Number tumors				
≤3	60.3	0.6	89.4	0.9
>3	52.7		87.5	
MC status				
IN	60.4	0.8	90.2	0.6
OUT	57.9		85.7	
UCSFC status				
IN	60.8	1.0	90.2	0.5
OUT	55.0		84.2	
LRT before				
LT No	58.3	0.3	87.5	1.0
Yes	59.8		89.5	
LT era				
1st era (1994–2000)	67.3	0.6	92.1	0.4
2nd era (2001–2010)	57.9		88.6	
Initial AFP				
≤200 ng/ml	*62.4*	*0.05*	*91.4*	*0.002*
>200 ng/ml	*37.0*		*69.3*	
Last AFP				
≤200 ng/ml	*64.0*	*<0.0001*	*91.7*	*<0.0001*
>200 ng/ml	*15.0*		*40.0*	
AFP slope				
≤15 ng/ml/month	*63.8*	*0.004*	*92.1*	*0.004*
>15 ng/ml/month	*30.0*		*59.7*	
Initial PLR				
≤150	59.5	0.6	90.0	1.0
>150	62.8		87.9	
Last PLR				
≤150	62.8	0.2	*91.6*	*0.02*
>150	49.7		*80.7*	
PLR slope				
≤2.9/month	58.3	0.5	87.7	0.3
>2.9/month	65.3		94.1	
Initial NLR				
≤5.4	59.9	0.6	90.2	0.4
>5.4	59.2		86.8	
Last NLR				
≤5.4	*64.5*	*0.02*	88.4	0.4
>5.4	*48.2*		92.6	
NLR slope				
≤0.1/month	57.4	0.6	88.1	0.9
>0.1/month	64.0		91.4	

Statistically significant values ($P < 0.05$) are reported in italic characters.

AFP, alpha-fetoprotein; ITT, intention to treat; LRT, loco-regional therapy; LT, liver transplantation; MC, Milan criteria; MELD, model for end-stage liver disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; TFS, tumor-free survival; UCSFC, University of California san Francisco criteria.

infiltrate negatively impacts on prognosis [31]. Recently, a complex system of pro-inflammatory cytokines and growth factors, released from the tumor itself or produced by the host as a reaction to the presence of a malignancy, has been identified to do this [32]. These findings were confirmed by molecular profiling, underlining the prognostic role of inflammation-related gene expression signatures in patients with HCC [33]. These findings may provide new insights in relation to the influence of, mainly intratumoral, inflammatory markers on tumor pathogenesis and progression. Moreover, such systemic inflammation can be expressed by different scores allowing prediction of the outcome of patients with HCC [17–24, 34].

Our group investigated the role of two inflammatory markers, NLR and PLR, as predictors of dropout, tumor-related dropout, death on the WL and post-LT recurrence in patients waiting for LT. To the best of our knowledge, the prognostic performance of these markers has so far not been studied in relation to the risk of dropout.

On c-statistics, the last NLR determination was the best prognostic test for the risk of dropout. Conversely, initial NLR value and NLR slope failed predict dropout. The last NLR of over 5.4 allowed good stratification of the population at risk of dropout. Interestingly, this variable was unable to predict the risk of post-LT recurrence. This result is not in agreement with other studies in which NLR was a predictor of recurrence [22–24]. Our data are corroborated by the pathological analyses of the hepatectomy specimen, in which similar poor grading and microvascular invasion rates were observed despite the 5.4 cutoff NLR value. A possible explanation for this observation might be due to the fact that higher NLR values corresponded to more advanced tumors, which were already selected out before considering LT. The majority of reported studies only analyzed the role of NLR in advanced tumors or in patients exceeding LT criteria [17–21]. In our study, indeed 43% of dropped-out patients (for any reason) had NLR values surpassing 5.4, while only 20.5% of such patients belonged to the group of transplanted patients. Dropped-out patients had larger tumors, higher number of lesions, higher AFP levels as well as higher inflammation values. Another explanation might be that NLR relates to the underlying (inflammatory) cirrhosis and that the risk of dropout is more due to the deteriorated liver function than to the tumor progression. The facts that the subanalysis aiming at identifying risk factors for tumor-related dropout was unable to sort out NLR as a significant predictor but in contrast as a

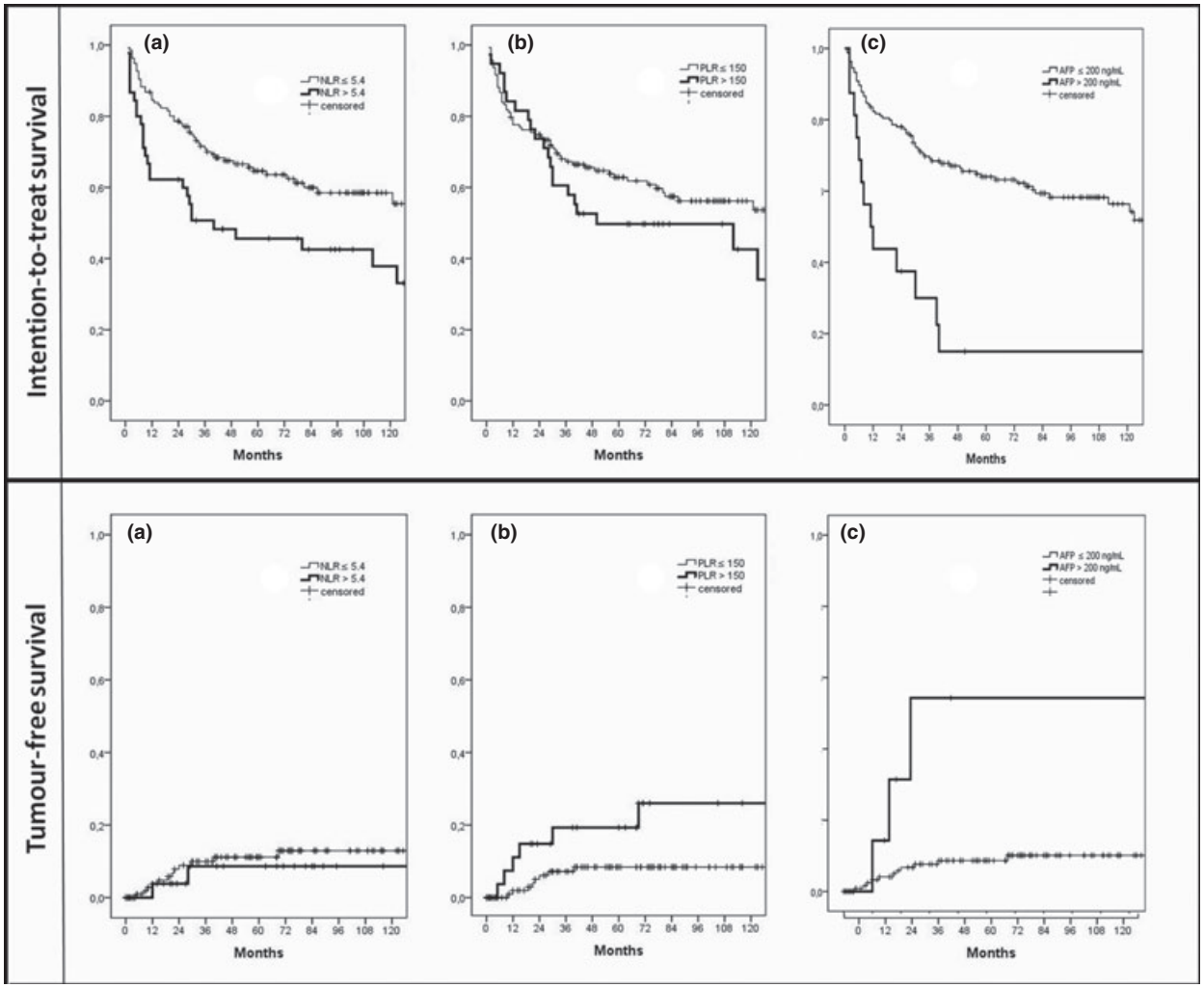

Figure 2 Intention to treat (ITT) and tumor-free survival (TFS) curves according to: (a) last NLR determination, (b) last PLR determination and (c) last AFP determination.

unique independent predictor for the risk of death during the waiting list goes along with this reasoning. It is clear that more detailed studies on larger patient cohorts are required to further define the role of this inflammatory parameter on tumor-related dropout and death during the waiting list, in order to analyze a greater number of events and to construct appropriate multivariate models. Comparing patients listed with HCC and non-HCC is also desirable to identify the role of NLR as a marker of worsening patient condition.

Conversely to NLR, PLR values played no role in selecting waiting list patients for LT, but the last PLR value permitted stratification of the patients in relation to the risk of recurrence. The prognostic role of PLR in patients with HCC has, until now, only been marginally investigated. Our results are in agreement with previous studies which showed that PLR was strongly linked to the risk of HCC recurrence [24, 34]. It is interesting to note that, in contrast to NLR, PLR is a good stratification tool only in a selected population of transplanted patients. PLR discriminates patients with microvascular invasion, a well-known risk factor for HCC recurrence. Patients with last pre-LT determination of over 150 had a 25% vascular invasion rate versus 9% in patients not exceeding this cutoff value. However, there is a possible selection bias in this setting. Despite the existing homogeneity in the evaluated population in relation to the disease status (Child-Turcotte-Pugh status C was observed in less than 15% of cases), it is difficult to say whether the platelet count was due more to an inflammatory than to a portal hypertensive status. A study in a large patient cohort corrected for the porto-systemic gradient is therefore advisable.

Table 4. ITT survival of inflammatory markers in the subgroups of HCV-infected, HBV-infected and noninfected patients.

	5-year ITT survival					
	HCV (n = 69)		HBV (n = 28)		Other causes (n = 79)	
Variables	%	*P*-value	%	*P*-value	%	*P*-value
Initial PLR						
≤150	56.0		64.6		61.1	
>150	73.3	0.62	100	0.36	57.1	0.74
Last PLR						
≤150	62.3		68.2		62.4	
>150	46.2	0.23	66.7	0.56	49.4	0.35
PLR slope						
≤2.9/month	54.8		66.3		60.1	
>2.9/month	73.0	0.14	71.4	0.68	57.1	0.87
Initial NLR						
≤5.4	59.7		65.8		58.4	
>5.4	55.6	0.52	75.0	0.83	60.6	0.80
Last NLR						
≤5.4	62.2		69.2		66.5	
>5.4	46.2	0.04	62.5	0.88	41.4	0.04
NLR slope						
≤0.1/month	55.2		68.8		55.6	
>0.1/month	66.0	0.40	63.6	0.28	66.2	0.26

ITT, intention to treat; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

The role of AFP as a well-known prognostic factor for HCC was confirmed in the present study [27, 35, 36]. The last AFP value was the best prognostic tool both for dropout before and recurrence after LT. High AFP values before LT correlated with poor histological findings such as poor grading and microvascular invasion.

This study showed, as previously reported, that the last NLR, PLR, and AFP values were stronger predictors than their initial and slope values. Clearly, the consideration of values taken exactly at the moment of dropout could reduce their usefulness in the clinical work-up, potentially transforming them into confirmative rather than predictive parameters. Unfortunately, the limitations of our study due to its retrospective design and the relatively small number of patients and events (i.e., dropout and recurrence) impact on the possibility of obtaining definitive conclusions on these data; larger, potentially prospective studies are needed to do so. The long time span (1994–2010) of the study could also be responsible for many confounding factors, such as pretreatment management, immunosuppressive regimens and diagnostic work-up, influencing the observed results. However, a subanalysis performed in the cohort of patients listed and transplanted during the period 2001–2010 confirmed the results that were drawn from the analysis of the entire patient cohort.

Looking at the possible conflicting role of hepatic viral infections versus cancer as possible inductors of liver inflammation, we observed that NLR maintained its discriminatory ability in the cohorts of HCV-infected and noninfected patients with HCC. In these latter patients, the effective increase in inflammatory markers seems more likely to be related to the effective role played by the cancer and not by viral-driven inflammatory mechanisms. In the 28 HBV-infected patients, no statistically significant results could be obtained due to the small sample size. It would be interesting to investigate that this latter aspect in a larger patient cohort presenting with an active replicative status. Unfortunately, the retrospective nature of the present study did not allow us to investigate the possible (linear?) correlation between different cirrhotic rates and inflammatory markers.

It should also be stressed that the studied patient cohort was submitted from the outset in 1994 to an aggressive cancer management policy as shown by the 93% incidence (82% in 1994–2000 and 96% in 2001–2010) of pre-LT LRT. It is difficult to answer how the differently applied LRTs impacted on inflammatory response, or if the NLR and PLR ratios calculated before and after LRT represent possible better selection criteria in respect to the analysis of their static values. In the present analysis, inflammatory marker slopes were not statistically significant at c-statistics and survival analyses, while last static values were. Moreover, median values of NLR and PLR slopes corresponded to the value of zero in both subgroups of dropout and transplanted patients, suggesting a potentially marginal role of LRTs in modifying them. A subanalysis focusing only on patients who had pre-LT TACE clearly confirmed not only the role of NLR as predictor of dropout and PLR as predictor of post-LT recurrence but also the superiority of the last values compared to the initially obtained ones or the slopes obtained before and after LRT.

Our intent was to investigate the role of inflammatory markers comparing them with some commonly adopted predictors of dropout and recurrence, which are available during the pre-LT period. Therefore, we did not take into consideration post-transplant variables, such as grading or microvascular invasion, demonstrated on pathological examination of the hepatectomy specimen, thereby accepting the potential bias related to such methodological approach.

Based on the internationally widely accepted 'morphological' (imaging) and the, in part presented here, 'biological' selection criteria of patients with HCC for LT, the time has come to set up and validate in a larger (multicentric) patient cohort a scoring system combining both types of parameters in order to refine the liver transplant inclusion criteria further. This is of special importance in patients with HCC surpassing the (MC and) UCSF Criteria.

In conclusion, the findings of this single center study have shown that the NLR may offer additional information in the clinical management of potential HCC liver recipients due to its predictive value in relation to risk of dropout on the waiting list and that PLR may add some value as a good predictor of risk for post-LT HCC recurrence. Besides these two inflammatory markers, AFP once more has been validated as a good predictor of both dropout before LT and recurrence after LT. The combination of classical (static) imaging and (dynamic) biological parameters, reflecting the aggressiveness of the tumor will represent a step forward in more adequately selecting potential HCC liver recipients and this possibly independently from their (Milan or UCSF) classification.

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Authorship

ECS, JL designed the research. QL, JMRJ and ECS collected the data. QL did the data analysis. QL, JL wrote the paper. All the authors did the critical evaluation of the paper.

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