Preoperative Inflammatory Markers as Prognostic Predictors After Hepatocellular Carcinoma Resection: Data From a Western Referral Center

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Abstract

Background

Recent studies from eastern centers have demonstrate an association between inflammatory response and long-term outcomes after hepatocellular carcinoma (HCC) resection. However, the prognostic impact of inflammatory markers in western patients, with distinct tumor and epidemiologic features, is still unknown.

Aim

To evaluate the prognostic impact of preoperative neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR), as well as their impact according to tumor size (< 5 cm, 5-10 cm, > 10 cm) in patients undergoing HCC resection with curative intent.

Methods

Optimal cut-off values for NLR, PLR, and MLR were determined by plotting the receiver operator curves. Overall survival (OS) and disease-free survival (DFS) curves were calculated using the Kaplan-Meier method and compared using the log-rank test. The Cox method was used to identify independent predictors of OS and DFS.

Results

In total, 161 consecutive adult patients were included. A high NLR (>1.715) was associated with worse OS (P=0.018). High NLR (>2.475; P=0.047) and PLR (>100.25; P=0.028) were predictors of short DFS. In HCC < 5 cm, MLR (>1.715) was associated with worse OS (P=0.047). In the multivariate analysis, high PLR was an independent predictor of worse DFS (hazard ratio [HR]=3.029; 1.499-6.121; P=0.002).

Conclusions

Inflammatory markers are useful tools to predict long-term outcomes after liver resection in western patients, high NLR was able to stratify subgroups of patients with short OS and DFS, an increased PLR was an independent predictor of short DFS, while high MLR was associated with short OS in patients with early HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary malignant liver tumor with increasing incidences in recent decades [1]. Currently, HCC is the third most frequent cause of cancer-associated mortality worldwide, with more than 900,000 deaths per year [2]. Among the curative modalities, resection is one of the mainstays of HCC treatment; however, the recurrence rate after liver resection remains high, reaching 50%-80% in 5 years [3].

The main prognostic factors for patients with HCC who underwent resection are serum alpha-fetoprotein levels, the number of lesions, tumor size, and the presence of vascular invasion, satellite nodules, and tumor pseudocapsule [4]. However, most of these factors can only be assessed after surgical specimen evaluation and cannot be used for preoperative patient selection. For this reason, the search for preoperative prognostic markers that may help understand the tumors' biology is advisable.

In recent years, several authors have shown that inflammation and immunologic responses are associated with the development of malignant tumors [5]. Briefly, the activation of the humoral response (mediated by neutrophils, platelets, and monocytes/macrophages) has an impact on carcinogenesis, favoring a pro-tumoral microenvironment and angiogenesis [5]. In contrast, the lymphocytic response, mediated by CD4+ and CD8+ T lymphocytes and natural killer (NK) cells, has an inhibitory effect on this process. In fact, cytokines produced by cellular response can mediate apoptosis and tumor cell death. Therefore, a dominant lymphocytic response could be associated with a better prognosis [6].

Recent studies have shown an association between inflammatory response and long-term outcomes in several solid gastrointestinal tumors [7, 8]. However, the prognostic impact of inflammatory markers in patients who underwent surgical resection for HCC is still under debate.

The neutrophil-to-lymphocyte ratio (NLR) is the most studied preoperative biomarker for patients with HCC. Several studies, mainly from eastern centers, have suggested that a high NLR may correlate with a poor prognosis in patients with HCC [9]. Moreover, recent studies have suggested that the NLR is also a prognostic factor in specific subgroups, such as patients with small tumors (< 5 cm) [10] or large HCCs (> 10 cm) [11]. However, other authors have failed to detect an association between NLR and HCC prognosis [12]. In recent years, a few eastern studies also suggested the impact of other inflammatory markers, such as the platelet-to-lymphocyte ratio (PLR) and monocyte-to-lymphocyte ratio (MLR), on long-term outcomes of HCC patients [6, 13].

Despite promising outcomes, few studies conducted in western centers, where HCC presents distinct tumor and epidemiologic characteristics, have assessed the ability of the inflammatory markers to predict long-term survival in patients with HCC undergoing liver resection [14]. Additionally, to our knowledge, no western studies have evaluated the impact of inflammatory markers on subsets of patients according to tumor size.

The primary endpoint of this study was to evaluate the prognostic impact of the NLR, PLR, and MLR on the long-term outcomes of patients who underwent curative hepatic resection for HCC. The secondary

endpoint was to evaluate the prognostic impact of these markers on subgroups of patients according to tumor size: < 5 cm, 5-10 cm, and > 10 cm.

2. Methods

This study was approved by the Institutional Ethics Committee of the Hospital das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, Brazil. (number: 3.004.022) and conducted according to the Standards for Reporting Studies of Diagnostic Accuracy (STARD) [15]. Patient consent form was not required (retrospective study).

From a prospective database, consecutive adult patients with pathologically proven HCC who underwent liver resection with curative intent between January 2007 and December 2018 were evaluated. The inclusion criteria were as follows: patients older than 18 years, uni or oligonodular disease (up to three nodules), and absence of extrahepatic disease. Patients with chronic liver disease and compensated liver function were considered eligible as follows: Child-Pugh A (or B7 when minor peripheral resection was required) and Model of End Stage Liver Disease (MELD) scores ≤ 10, without clinically significant portal hypertension (small caliber esophageal varices and platelets > 100.000/mL), and future liver remnant ≥ 40% [16]. The exclusion criteria were as follows: presence of extrahepatic disease, R1/R2 resection, previous systemic or locoregional treatment addressed to HCC, presence of infection, and use of preoperative antibiotics or corticosteroids.

All patients underwent clinical evaluation and laboratory tests for liver function. Preoperative workup included abdominal helicoidal computed tomography (CT) or magnetic resonance imaging (MRI), and thoracic CT. Preoperative diagnosis was based on image characteristics; biopsy was only indicated if diagnostic doubt persisted after radiologic evaluation. When CT or MRI showed signs of portal hypertension, upper digestive endoscopy was performed. Surgery was performed after a multidisciplinary meeting discussion.

The following preoperative characteristics were studied: age, sex, body mass index (BMI), preoperative laboratory tests, etiology of chronic liver disease, size and location of the lesions, presence of cirrhosis, and portal hypertension. Inflammatory markers were evaluated within seven days of surgery. The NLR was calculated by dividing the absolute neutrophil count (number of neutrophils/mL) by the absolute lymphocyte count (number of lymphocytes/mL); the PLR was calculated by dividing the absolute platelet count (number of platelets/mL) by the absolute lymphocyte count (number of lymphocytes/mL); and the MLR was calculated by dividing the absolute monocyte count (number of monocytes/mL) by the absolute lymphocyte count (number of lymphocytes/mL).

For the intra- and postoperative periods, the following data were retrieved: blood transfusion requirement, length of stay in the intensive care unit (ICU), length of hospital stay, perioperative complications, overall survival (OS), and disease-free survival (DFS). The specimens obtained were assessed for the number of nodules, size of the larger nodule (in millimeters [mm]), degree of tumor differentiation (histological grade), presence of satellite lesions, and presence of vascular invasion.

Perioperative morbidity was defined as any event occurring during the first 90 postoperative days. OS was defined as the time interval between liver resection and the date of death or the most recent follow-up date if the patient was alive. DFS was defined as the time interval between liver resection and recurrence at any site or the most recent follow-up date.

2.1. Statistical analysis

Continuous data were expressed as median and interquartile range or mean ± standard deviation. Categorical variables were expressed as percentages. Quantitative data were compared using the t-test or Mann-Whitney U-test, as appropriate. For categorical variables, Fisher's exact test or the chi-squared test was used.

The optimal cut-off values for the NLR, PLR, and MLR were calculated using receiver operator curves (ROC) and Youden's index. Thereafter, the patients were divided into two groups: below and above the calculated cut-offs. OS and DFS were estimated using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were used to identify predictors associated with OS and DFS. Statistical significance was set at 5%.

3. Results

3.1. Baseline characteristics

During the study period, 207 patients with histologically confirmed diagnosis of HCC underwent liver resection; one patient (0.5%) was younger than 18 years, 12 (4.8%) patients underwent preoperative transarterial chemoembolization (TACE) or radiofrequency ablation, nine patients (4.3%) had a preoperative MELD > 10, 18 (8.7%) patients underwent R1 resections, and six (2.9%) presented signs of infection or the use of antibiotics immediately before the surgery. After applying the exclusion criteria, 161 patients were enrolled in the study. The baseline characteristics of the patients are summarized in Table 1. The main causes of chronic liver disease were hepatitis C (60%), hepatitis B (20%), nonalcoholic steatohepatitis (NASH, 11%), alcoholic liver disease (5%), and other etiologies (4%).

Table 1
Baseline characteristics of the included patients (N=161)

Age (years)	**62 ± 11**
mean ± SD	**63 (18-86)**
median (min-max)	
Sex (%)	108 (67.1%)
male	53 (32.9%)
female	
BMI (kg/m^2)	25.4 ± 4.5
mean ± SD	24.9 (22.6-27.7)
median (quartile 25-75)	
Cirrhosis (%)	135 (83.9%)
yes	26 (16.1%)
no	
Child-Pugh (%)[†]	111 (82.2%)
A5	17 (12.6%)
A6	7 (5.2%)
B7	
Preoperative MELD	8 ± 3
mean ± SD	8 (7-9)
median (quartile 25-75)	
Portal hypertension (%)	43 (26.7%)
yes	92 (73.3%)
no	
Esophageal varices (%)	22 (13.7%)
yes	21 (86.3%)
no	

SD: standard deviation; BMI: body mass index; MELD: Model for End-Stage Liver Disease; INR: international normalized ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio

[†] % of patients with cirrhosis

Age (years)	**62 ± 11**
mean ± SD	**63 (18-86)**
median (min-max)	
Hemoglobin (g/dL)	13.7 ± 3.8
mean ± SD	13.7 (12.6-14.9)
median (quartile 25-75)	
Platelet count (/mm^3)	186410 ± 97208
mean ± SD	170000 (118000-230000)
median (quartile 25-75)	
Bilirubin (g/dL)	0.72 ± 0.22
mean ± SD	0.65 (0.47-0.89)
median (quartile 25-75)	
Aspartate aminotransferase (AST, U/L)	62.0 ± 61.0
mean ± SD	42.0 (28.0-68.0)
median (quartile 25-75)	
Alanine aminotransferase (ALT, U/L)	54.7 ± 51.0
mean ± SD	38.0 (25.0-69.0)
median (quartile 25-75)	
INR	1.1 ± 0.1
mean ± SD	1.1 (1.0-1.2)
median (quartile 25-75)	
Creatinine (mg/dL)	1.0 ± 1.0
mean ± SD	0.9 (0.7-1.1)
median (quartile 25-75)	

SD: standard deviation; BMI: body mass index; MELD: Model for End-Stage Liver Disease; INR: international normalized ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio

† % of patients with cirrhosis

Age (years)	**62 ± 11**
mean ± SD	**63 (18-86)**
median (min-max)	
Alpha-fetoprotein (ng/mL)	2483.1 ± 9906.5
mean ± SD	19.0 (4.7-172.7)
median (quartile 25-75)	
Albumin (g/dL)	4.0 ± 0.3
mean ± SD	4.1 (3.7-4.5)
median (quartile 25-75)	
Neutrophil count (/mm^3)	3601 ± 3465
mean ± SD	3300 (2300-4410)
median (quartile 25-75)	
Lymphocyte count (/mm^3)	1869 ± 773
mean ± SD	1700 (1300-2300)
median (quartile 25-75)	
Monocyte count (/mm^3)	575 ± 308
mean ± SD	510 (400-700)
median (quartile 25-75)	
NLR	2.3 ± 2.2
mean ± SD	1.9 (1.4-2.6)
median (quartile 25-75)	
PLR	115.4 ± 89.4
mean ± SD	96.2 (67.0-144.4)
median (quartile 25-75)	

SD: standard deviation; BMI: body mass index; MELD: Model for End-Stage Liver Disease; INR: international normalized ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio

† % of patients with cirrhosis

Age (years)	**62 ± 11**
mean ± SD	**63 (18-86)**
median (min-max)	
MLR	3.8 ± 2.0
mean ± SD	3.5 (2.4-4.6)
median (quartile 25-75)	
Tumor size (mm)	62.0 ± 50.7
mean ± SD	42 (29.0-80.0)
median (quartile 25-75)	
Tumor grade (%)	9 (5.6%)
well differentiated	104 (64.6%)
moderately differentiated	28 (17.4%)
poor differentiated	20 (12.4%)
unavailable	
Satellite nodules (%)	40 (24.8%)
yes	121 (75.2%)
no	
Vascular invasion (%)	82 (50.9%)
yes	75 (43.8%)
no	4 (2.5%)
unavailable	
SD: standard deviation; BMI: body mass index; MELD: Model for End-Stage Liver Disease; INR: international normalized ratio; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio	
† % of patients with cirrhosis	

The OS of the entire cohort was 65.2% at 3 years, 47.6% at 5 years and 28.4% at 10 years, while DFS was 61.1% at 3 years, 44.4% at 5 years and 20.1% at 10 years (Supplementary Figure 1).

3.2. Optimal cut-offs for NLR, PLR and MLR

The cut-off values of the inflammatory markers were determined by plotting the ROC curves for mortality and recurrence after resection. The best cut-offs calculated using the Youden index are listed in Table 2.

Table 2
Diagnostic accuracy of the calculated cut-offs for mortality and recurrence

Mortality						
	Cut-off	**Sensibility**	**Specificity**	**1 - Specificity**	**LR+**	**LR-**
NLR	> 1.715	0.639	0.483	0.517	1.236	0.747
PLR	> 115.050	0.375	0.697	0.303	1.236	0.897
MLR	> 1.750	0.917	0.112	0.888	1.033	0.742
Recurrence						
	Cut-off	**Sensibility**	**Specificity**	**1 - Specificity**	**LR+**	**LR-**
NLR	> 2.475	0.307	0.732	0.268	1.146	0.947
PLR	> 100.250	0.520	0.620	0.380	1.367	0.775
MLR	> 2.680	0.747	0.310	0.690	1.082	0.818

NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio; LR+: positive likelihood ratio; LR-: negative likelihood ratio

The NLR, PLR, and MLR areas under the curve (AUC) for mortality were 0.541 (95%CI 0.451-0.631), 0.479 (95%CI 0.388-0.571), and 0.454 (95%CI 0.365-0.543), respectively. Regarding recurrence, the calculated AUC were 0.479 (95%CI 0.385-0.573), 0.519 (95%CI 0.424-0.614), and 0.469 (95%CI 0.372-0.565), respectively.

3.3. Prognostic value of inflammatory markers for OS and DFS

A high NLR (> 1.715) was a predictor of short OS in patients who underwent HCC resection. The 5-year OS was 56% in the low NLR group and 40% in the high NLR group (P=0.018, Figure 1).

Clinicopathological characteristics of patients with low (≤ 1.715) and high NLR (> 1.715) are summarized in Supplementary Table 1. Patients with high NLR had lower serum albumin levels (4.1 g/dL [3.7-4.5] vs. 4.3 g/dL [4.1-4.6]; P=0.028) and larger tumors (77 mm [35-100] vs. 39 mm [21–45]; P < 0.001) and were associated with higher values of PLR (134 [91.2-160] vs. 72.4 [53.7-93.2]; P < 0.001) and MLR (4.4 [3.4-5.5] vs. 3.1 [2-3.8]; P < 0.001).

Patients with high NLR (> 2.475) presented higher total bilirubin levels (0.7 g/dL [0.5-0.9] vs. 0.6g/dL [0.5-0.7]; P=0.020) and larger tumors (67 mm [40-100] vs. 40 mm [25-65]; P=0.003) when compared to

patients with low NLR. There was also an association with high PLR (147.2 [104.5-176] vs. 82.3 [60-108]; P < 0.001) and high MLR (3.8 [3-5.2] vs. 2.1 [1.6-3.3]; P < 0.001) (Supplementary Table 2).

Patients with high PLR (> 100.25) presented higher serum levels of total bilirubin (0.7 g/dL [0.5-0.9] vs. 0.6 g/dL [0.4-0.8]; P=0.004), larger tumors (75 mm [40-125] vs. 34 mm [22–45]; P < 0.001), and a higher frequency of vascular invasion (62.1% vs. 42%; P=0.020). Additionally, an association with higher values of NLR (2.5 [1.9-3.5] vs. 2.5 [1.9-3.6]; P < 0.001) and MLR (4 [3-5.2] vs. 3.1 [1.8-3.8]; P < 0.001) were observed (Supplementary Table 3).

3.4. Risk factors for OS and DFS after hepatectomy

Variables associated with OS and DFS after HCC resection on univariate analysis are shown in Table 3.

Table 3
Univariate analyses of prognostic factors associated with overall and disease-free survival

Overall survival			Disease-free survival		
Variable	**P**	**HR IC95%**	**Variable**	**P**	**HR IC95%**
Hepatitis C	0.016	1.98 (1.13-3.40)	**Satellites nodules**	0.023	1.77 (1.08-2.92)
Portal Hypertension	0.005	2.16 (1.25-3.74)	**Vascular invasion**	0.005	1.97 (1.22-3.19)
Esophageal varices	0.047	1.90 (1.10-3.60)	**Age > 50 years**	0.050	0.54 (0.95-1.00)
Transfusion	0.002	2.38 (1.38-4.10)	**Bilirubin > 1.2 mg/dL**	0.034	2,41 (1,15-5,07)
Perioperative complications	0.006	2.00 (1.21-3.34)	**AST > 50 U/dL**	0.020	1.63 (1.10-2.59)
Vascular invasion	0.007	2.02 (1.20-3.40)	**Alpha-fetoprotein > 20 ng/ml**	< 0.001	3.64 (2.23-5.91)
Bilirubin > 1.2 mg/dL	0.048	1.90 (1.05-3.60)	**NLR > 2.475**	0.047	1.28 (1.01-1.96)
AST > 50 U/dL	0.021	1.85 (1.09-3.14)	**PLR > 100.25**	0.028	1.60 (1.02-2.52)
ICU stay > 3 days	<0.001	3.06 (1,80-5,23)			
Alpha-fetoprotein > 20 ng/ml	<0.001	3.42 (1.96-5.91)			
NLR > 1.715	0.018	1.61 (1.01-2.67)			

AST: aspartate aminotransferase; ICU: intensive care unit; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio

Multivariate analysis showed that the presence of portal hypertension (HR=7.035; 95%CI 2.366-20.660; P < 0.001), a preoperative aspartate aminotransferase (AST) level > 50 U/dL (HR=3.057; 95%CI 1.104-8.470; P=0.032), and an ICU stay > 3 days (HR=5.039; 95%CI 1.752-14.489; P=0.003) were independent predictors of short OS. Regarding DFS, AST level > 50 U/dL (HR=3.324; 95%CI 1.600-6.905; P=0.001), presence of vascular invasion (HR=2.363; 95%CI 1.133-4.928; P=0.022), and high PLR (HR=3.029; 95%CI 1.499-6.121; P=0.002) were predictors of a high recurrence rate.

3.5. Subgroup analysis

Survival analysis was also performed in patients with HCC according to tumor size: < 5 cm (group 1, N=98), 5-10 cm (group 2, N=35), and > 10 cm (group 3, N=28).

A high MLR (> 1.750) was a predictor of short OS in group 1 (P=0.047), while the NLR and PLR were not good predictors of OS (Supplementary Figure 2). None of the inflammatory markers were able to predict DFS in this subset of patients (Supplementary Figure 3). In groups 2 and 3, the NLR, PLR, and MLR were not able to predict OS or DFS (Supplementary Figures 4-7).

4. Discussion

Cancer is characterized by neoplastic cells that are closely related to chronic inflammatory infiltrates. In fact, there is increasing evidence showing that systemic inflammation is responsible for tumor-promoting activities and neoangiogenesis in several tumors [17].

Humoral response has an impact on carcinogenesis, exerting pro-tumoral action [18]. Recent studies have shown that the molecular environment created by humoral response (mediated by the production of enzymes such as metalloproteinases, vascular growth factors, and cytokines) favors conjunctive matrix degradation, the activation of neoangiogenesis, the recruitment and activation of cell profiles favoring tissue invasion, and metastasis. Therefore, an increase in humoral inflammatory response can lead to worse oncological outcomes [18]. Conversely, lymphocytic cellular response (mediated by T lymphocytes CD4+, CD8+, and NK cells) inhibits carcinogenesis, leading to better oncological prognosis [17].

Recent studies have shown the interaction between platelets and tumoral microenviroment [19]. The main platelet-associated mechanisms are based on signaling pathways that orchestrate tumor growth, activation of angiogenesis, and metastatic dissemination [20]. In fact, platelets play a pivotal role in tumor neoangiogenesis from the early to advanced stages [21]. Activated circulating platelets have metabolic pathways diverted to synthesize vascular growth factors such as vascular endothelial growing factor and platelet-derived growing factor, which leads to changes in vascular permeability and in the underlying smooth musculature [21]. Activated platelets also take part in the metastatization process through the activation of the epithelium-mesenchymal transition, an initial phenomenon in metastasis development [22]. Additionally, activated circulating platelets bind to tumor cells, acting as a mechanical protection against immune cytotoxic cells, especially NK cells [23].

The prognostic impact of systemic inflammatory response has been studied in several gastrointestinal tumors, such as pancreatic, colorectal, and gastric cancers [24, 25]. The main advantages of inflammatory markers include calculation using routine laboratory tests, low cost, and access to results before therapeutic intervention [26].

The NLR is the most studied inflammatory index. A large metanalysis, comprising more than 40,000 patients showed an association of high NLR with lower survival rate in patients with several solid tumors [27]. Recently, a study from our institution conducted by Szor et al [7]. showed that the NLR had an impact on OS (HR=1.50; 95%CI 1.27-4.21; P=0.048) and refines the TNM staging in patients with gastric

adenocarcinoma who underwent curative intent gastrectomy. In a systematic review, Malietzis et al. [28] showed that a high NLR had a negative impact on OS and DFS in patients with colorectal cancer undergoing resection. Likewise, Yshibashi et al. [29] studied patients undergoing curative intent esophagectomy for cancer and showed a close correlation between the NLR and OS.

However, the prognostic impact of inflammatory markers in patients with HCC who undergo resection remains controversial. Most studies that assessed these prognostic markers came from eastern centers, where HCC presents distinct clinical and epidemiological features [30]. The present study is one of the first from a western center to evaluate the association between main inflammatory markers (NLR, PLR, and MLR) and long-term outcomes after liver resection for HCC. In our study, the mean age was 62 ± 11 years, similar to those in other western centers but higher than those in eastern centers (52 ± 9 years) [31]. Regarding chronic liver disease etiology, hepatitis C (60%) was the most frequent, followed by hepatitis B (20%) and NASH (11%). In contrast, in eastern centers, the prevalence of hepatitis B infection is higher than 50% [32]. Our data showed that 84% of patients had chronic liver disease and 94.8% were classified as Child-Pugh A. Beard et al. [33] compared surgical outcomes after HCC resection in cirrhotic and non-cirrhotic North American patients and found a cirrhosis prevalence of 73%. In the eastern centers, the prevalence of cirrhosis/chronic liver disease is lower than 54% [9].

In our study, the median alpha-fetoprotein value was 19 ng/mL (4.7-172.7), while in eastern studies, it was 46.55 ng/mL (15.01-369.47) [9]. Several studies have demonstrated the correlation between alpha-fetoprotein levels and tumor volume or the presence of satellite lesions [34]. In fact, eastern studies showed larger tumors and a higher frequency of satellite nodules [35]; thus, the lower median AFP values in our study were probably due to a lower tumor burden.

The preoperative NLR is the most studied biomarker in patients with HCC. Although several studies have suggested that high NLR may correlate with a poor prognosis [9, 10], others failed to detect this association [12]. Another issue is that the reported cut-off values of NLR were different across the studies, which could not provide a consistent standard for comparison among different populations. Additionally, some studies have used the same cut-off for OS and DFS [36].

OS and DFS represent different aspects of disease evolution and treatment; therefore, different cut-offs for each outcome are advisable. The present study showed that a high NLR (> 1.715) was a predictor of short OS in patients undergoing hepatectomy for HCC. The 5-year OS was 56% in the low NLR group and 40% in the high NLR group (P=0.018). Similarly, NLR > 2.745 was also a predictor of short DFS (p=0.047). A systematic review including 100 studies (40,559 patients) showed that a high NLR was associated with adverse outcomes in several solid neoplasms, including HCC [27]. Similarly, in a recent meta-analysis conducted by Xingshun et al. [37] including 20,475 patients with HCC, it was found that patients with low NLR (< 2.0) presented better OS when compared to patients with high NLR (> 4.0) (HR=1.80; 95%CI 1.59-2.04; P < 0.00001).

However, in the multivariate analysis, the NLR was not an independent factor associated with OS or DFS in our study, which was also observed in other studies, especially from western centers. In a study

conducted by Sullivan et al. [12], evaluating patients with HCC showed that the NLR was not a predictor for OS after surgical or locoregional treatment (HR=1.09; 95%CI 0.95-1.24; P=0.23). Another study from the United Kingdom showed that the NLR was a predictor of DFS (HR=4.67; 95%CI 1.88-11.64; P=0.001) but was not a good predictor of OS in cirrhotic patients undergoing HCC resection. Interestingly, no relationship was found between NLR and prognosis in non-cirrhotic patients [38]. Thus, the presence of cirrhosis may impact the predictive value of NLR, justifying the heterogeneous results between the available studies.

Few studies have addressed the prognostic impact of other inflammatory markers in HCC patients [39]. In our study, we observed that high PLR (> 100.25) was an independent factor of DFS, which is consistent with recent studies [40]. Kaida et al. [41] evaluated patients with early-stage HCC who underwent resection and compared five inflammatory marker scores, showing that preoperative PLR was an independent predictor of recurrence. Similarly, Qing et al. [40] showed that increased preoperative platelet levels were associated with a higher recurrence rate following HCC resection. Thus, there is a potential association between platelet-derived markers and the prognosis of patients with HCC who underwent liver resection.

To date, few studies have evaluated the prognostic impact of MLR in HCC patients [42]. An interesting finding of our study was the prognostic impact of MLR on OS in patients with early-stage HCC (< 5 cm). This finding can be justified by the fact that activation of monocytes and macrophages usually occurs at earlier stages of tumor growth. Otherwise, in patients with larger lesions, other cells such as neutrophils and platelets play a predominant role in local invasion and metastatic dissemination [43].

Another independent factor associated with short OS in the present study was the presence of portal hypertension, which is in accordance with other studies [44]. In a meta-analysis comprising 2,285 HCC patients who underwent resection, the group of patients with portal hypertension presented short OS than the group without portal hypertension (HR=1.48; 95%CI 1.11-1.98; P=0.007) [45]. An AST level > 50 U/dL was an independent factor related to both OS and DFS. The exact mechanism underlying this finding is poorly understood; however, it might be explained by the fact that AST is exclusively present in hepatocytes and released into the circulation during liver inflammatory insults. Additionally, the reduced clearance in progressive chronic hepatic disease can lead to an increase in AST levels [46]. In our study, microvascular invasion was also an independent prognostic factor for recurrence. In fact, vascular invasion is frequently associated with higher recurrence rates due to aggressive biological behavior, represented by a greater volume of micrometastatic disease and a higher frequency of mural invasion [47].

This study is one of the first studies from a western center to evaluate the impact of the main inflammatory markers on the long-term outcomes after HCC resection, as well as in subgroups stratified by tumor size. Based on our findings, all the studied inflammatory markers are useful tools to predict long-term outcomes after liver resection in western patients. High NLR was able to stratify subgroups of patients with short OS and DFS, and increased PLR was a marker of short DFS, while high MLR was

associated with short OS in patients with early HCC. In fact, these markers were able to identify subgroups of patients with poor clinical features, such as higher bilirubin levels, larger tumors, and a higher frequency of vascular invasion. Therefore, inflammatory indexes are promising tools for preoperative selection of patients who require strict postoperative follow-up or even potential candidates for new adjuvant strategy protocols.

However, our findings should be viewed with caution due to some limitations. The first was the retrospective nature of this study, which increases the risk of selection, confusion, and measurement biases. Another limitation was the small number of patients enrolled, which may impair statistical power, especially in the subgroup analysis. Thus, the insights provided herein should be confirmed by larger prospective studies.

In conclusion, our study suggested that a high preoperative NLR is associated with short OS and DFS, whereas a high PLR is an independent factor associated with short DFS. In the subset of patients with HCC < 5 cm, a high MLR is a predictor of short OS.

List Of Abbreviations

HCC: hepatocellular carcinoma

NK: natural killer cells

NLR: neutrophil-to-lymphocyte ratio

PLR: platelet-to-lymphocyte ratio

MLR: monocyte-to-lymphocyte ratio

STARD: Standards for Reporting Studies of Diagnostic Accuracy

MELD: Model for End-Stage liver Disease

CT: computed tomography scan

MRI: magnetic resonance imaging

BMI: body mass index

ICU: intensive care unit

OS: overall survival

DFS: disease-free survival

ROC: receiver operator curves

TACE: transarterial chemoembolization

NASH: non-alcoholic steatohepatitis

SD: standard deviation

AST: aspartate aminotransferase

ALT: alanine aminotransferase

INR: international normalized ratio

LR+: positive likelihood ratio

LR-: negative likelihood ratio

AUC: area under the curve

Declarations

Ethics approval statement: This study was approved by the Ethics Committee of the Hospital das Clinicas, University of Sao Paulo School of Medicine, Sao Paulo, Brazil. (number: 3.004.022)

Patient consent statement: patient consent form was not required (retrospective study)

Permission to reproduce material from other sources: not applicable

Consent for publication: not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files]

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Author's Contributions: J.P.M.S., F.F.C., A.J.F.C. and P.H. wrote the main manuscript text. G.M.F., V.B.J., J.D.M.J. and S.C.N. prepared all figures. All authors have read and approved the manuscript.

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Figures

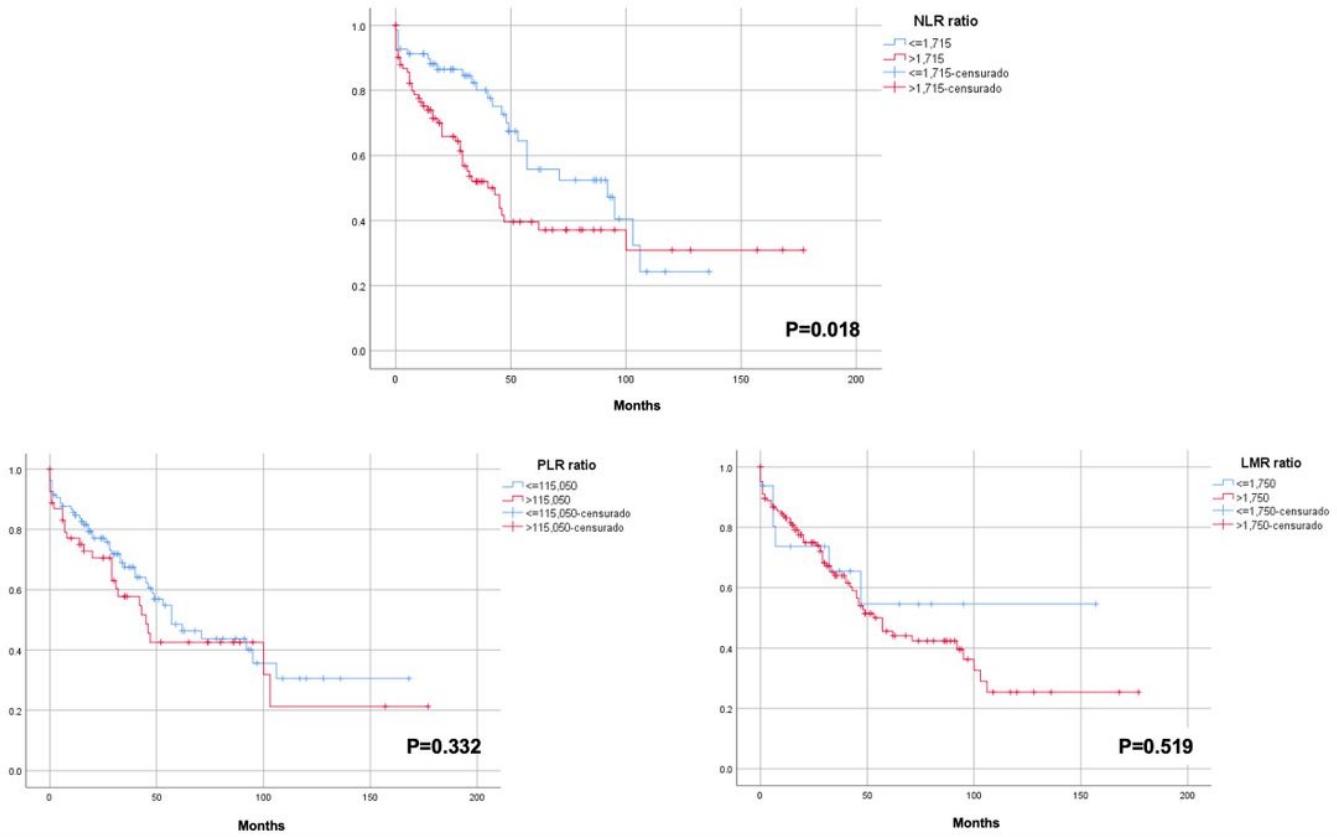

Figure 1

Overall survival of hepatocellular carcinoma patients with low (blue) and high (red) neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR)

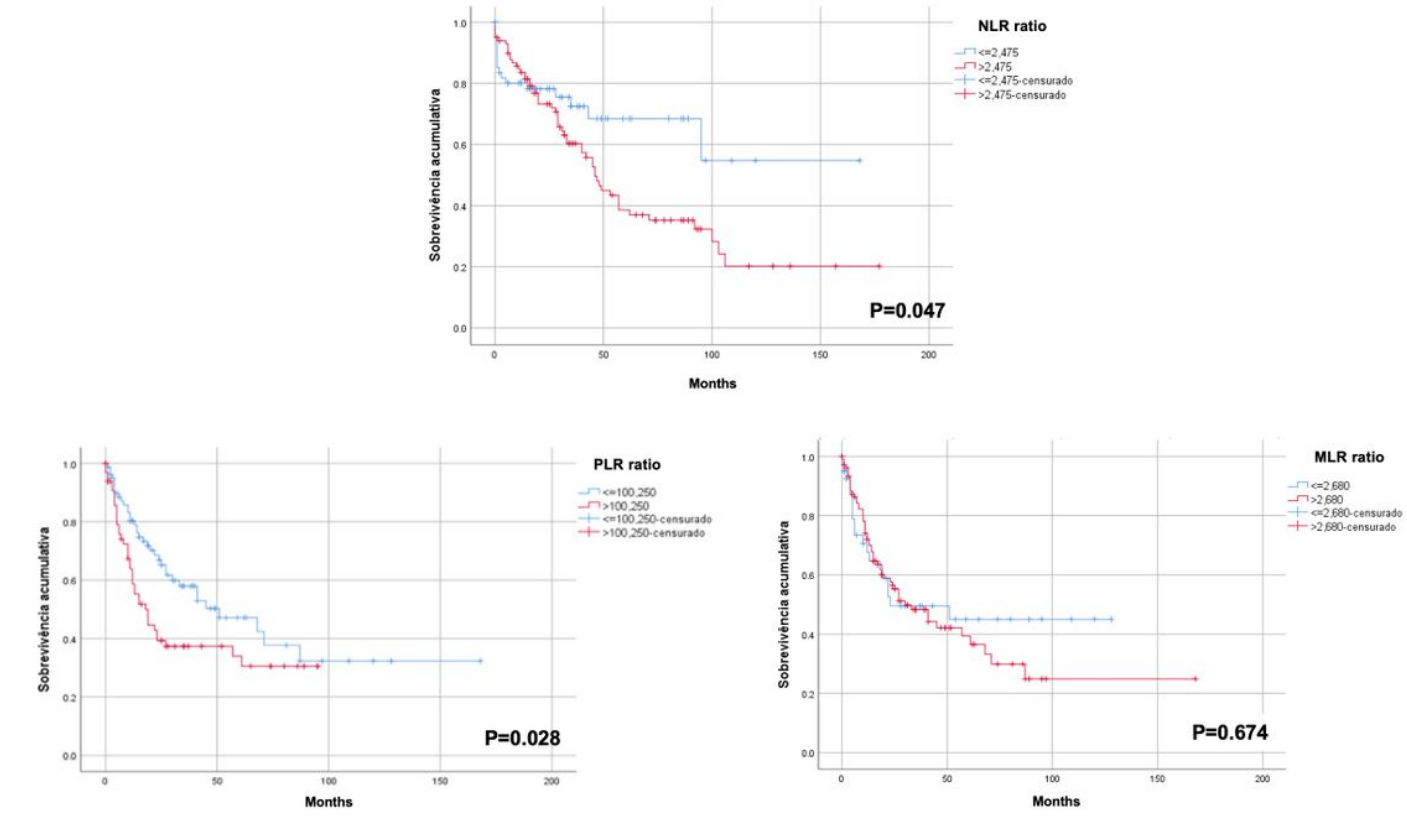

Figure 2

Disease-free survival of hepatocellular carcinoma patients with low (blue) and high (red) neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR)

Supplementary Files

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