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Association of inflammatory biomarkers with long-term outcomes after curative surgery for mass-forming intrahepatic cholangiocarcinoma

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Key words: Inflammatory biomarker, intrahepatic cholangiocarcinoma, long-term outcome

Abstract

Purpose: Inflammatory biomarkers such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) are reportedly predictive of long-term outcomes in several cancers. We evaluated their correlations with post-surgical long-term outcomes in patients with mass-forming (MF) intrahepatic cholangiocarcinoma (ICC).

Methods: We included 52 patients who underwent hepatic resection for MF-ICC at our hospital. We determined cutoff values of NLR, LMR and PLR, using receiver operating characteristics curves, and compared survival rates of patients with high- and low values. We also evaluated a prognostic scoring system based on significant inflammatory biomarkers.

Results: Cutoff values were determined as NLR: 1.93, LMR: 4.78, and PLR: 98. The high-NLR and low-LMR groups had significantly worse prognoses than the low-NLR and high-LMR groups, respectively. We therefore designed a scoring system (inflammation score [IS]) based on NLR and LMR values that stratified patients into three groups (scores 0, 1, or 2). IS was significantly correlated with overall survival

(OS; 5-year survival rates by IS score—0: 100%, 1: 61%, 2: 32%; $P = 0.011$), and shown to be an independent predictor of OS in multivariate analysis.

Conclusions: The IS system may predict long-term outcomes after surgery for MF-ICC.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer after hepatocellular carcinoma (HCC), and is commonly treated with surgical resection when possible [1, 2, 3]. However, recurrence rates after ICC surgery are high; 5-year recurrence-free survival is only 2%–39%, and overall survival is also poor; 5-year overall survival (OS) is reportedly only 5%–56% [4].

Inflammatory biomarkers, such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR), have been widely studied as prognostic markers for long-term outcomes in several cancers [5, 6, 7]. These biomarkers are also reportedly significant predictors of long-term outcome after ICC surgery [8, 9, 10]. However, these studies rarely consider the macroscopic types of ICC, which are reported to have different prognoses after surgery between each type [11, 12, 13, 14, 15, 16, 17].

This study evaluated correlations between inflammatory biomarkers and long-term outcomes in the patients who underwent surgery for the mass-forming (MF) subtype of ICC. We limited our cohort to MF-ICC to reduce its heterogeneity due to

different macroscopic types, and because MF is reported to be the predominant ICC subtype [18, 19]. We also evaluated a scoring system for comprehensive inflammatory status based on plural inflammatory biomarkers.

Methods

Patients

Patients who underwent hepatic resections for ICC at our hospital between May 1998 and May 2017 were eligible for this study. We excluded patients who (a) underwent preoperative therapies such as radiotherapy or chemotherapy; (b) had other malignant disease; (c) underwent bile-duct reconstructions; (d) died within 30 days after surgery due to postoperative complications; (e) underwent non-curative resections; (f) had combined hepatocellular-cholangiocarcinoma. This study was approved by the institutional review board of the Graduate School of Medical Sciences, Kyushu University (No. 30-578). All study participants were provided with the opportunity to opt out.

Surgical procedure and postoperative follow-up strategy

The details of our surgical techniques and patient follow-up methods for ICC have been reported previously [14, 20, 21]. Basically, anatomical hepatic resection was performed in patients who had adequate postoperative remnant liver volume and function. For patients with cirrhosis, or who appeared unlikely to have adequate liver volume after surgery, parenchymal-sparing hepatectomy was selected. Lymphadenectomy around the hepatoduodenal ligament was performed in patients whose preoperative imaging studies and intraoperative findings indicated possible lymph node metastasis.

After discharge, all patients underwent regular screening for recurrence, using ultrasonography and tumor markers such as CEA and CA19-9. In addition, computerized tomography scanning was performed every six months. If recurrence was suspected, additional imaging studies such as magnetic resonance imaging were performed. When ICC recurrence was confirmed with imaging studies, patients underwent additional hepatectomy or systemic chemotherapy, according to the size, site and number of tumors, and the patients' general condition.

The administration of postoperative adjuvant chemotherapy in this setting was determined by the physician's decision because consensus on the benefit of adjuvant chemotherapy for ICC patients is lacking [22]. Physicians decided the appropriateness of adjuvant chemotherapy by considering pathological findings and patient's general condition. Gemcitabine hydrochloride was used in the regimen.

Inflammatory biomarkers

Data on inflammatory biomarkers, including NLR, LMR, and PLR, were obtained from preoperative complete blood counts (CBC). NLR, LMR, and PLR were calculated as the absolute counts of neutrophils, lymphocytes, and platelets divided by the absolute counts of lymphocytes, monocytes, and lymphocytes, respectively. We used preoperative CBC data taken when patients showed no sign of infection. Cutoff values of these markers were determined using receiver operating characteristics (ROC) curves.

As a comprehensive evaluation of inflammatory status, we assessed a scoring system using inflammatory biomarkers that were significantly correlated with long-term outcomes.

Outcomes and statistical analysis

Patient data (including clinicopathological characteristics, laboratory data, operative findings, pathological findings, and survival data) were obtained from a prospectively maintained institutional database. Tumor stages were assessed according to the American Joint Committee on Cancer (AJCC) classification system, 8th edition. Active hepatitis B or C were defined as seropositivity for hepatitis B surface antigen or hepatitis C antibody, respectively. Alcoholic hepatitis and non-alcoholic steatohepatitis were defined considering patients' social histories and pathological findings of non-cancerous parts of surgical specimens. If the non-cancerous surgical sample showed F4-stage fibrosis, the patient was defined as having cirrhosis.

The patients were divided into high- and low-value groups for each inflammatory biomarker. Characteristics and survival rates of high- and low-value groups were compared.

Statistical analysis

Statistical analyses were performed using Wilcoxon rank-sum test for examining differences in continuous variable distributions, and Fisher's exact test for

categorical variables. Survival curves were analyzed using the Kaplan–Meier method and compared using the log-rank test. The inflammatory biomarkers that were significantly associated with survival rates were used to calculate the score to evaluate patients' comprehensive inflammation conditions; the score was designated as the inflammation score (IS). The usefulness of IS was assessed using the Kaplan–Meier method and log-rank test.

The Cox proportional hazards model was used for univariate and multivariate survival analyses. Factors, including IS, that were significantly associated with survival rates in univariate analyses were included in the multivariate analyses to assess their independence. $P< 0.05$ was considered significant. All of the analyses were conducted using JMP software (SAS Institute, Cary, NC).

Results

Sample size and inflammatory biomarker values classified by macroscopic types

We identified 52 patients with MF-ICC, 7 with MF+periductal infiltrating (PI)-ICC, 4 with PI-ICC, and 1 with intraductal growth (IG)-ICC who met our inclusion criteria. A flowchart of patient inclusion and exclusion is shown in Figure 1. Mean

values of inflammatory biomarkers by macroscopic type are shown in Table 1. In the following study, we analyzed only MF-ICC cases.

Patient characteristics and inflammatory biomarker cutoff values

We based our ROC curves on mortality at 5 years after surgery (Figure 2). The cutoff values of inflammatory biomarkers were NLR: 1.93, LMR: 4.78, and PLR: 98. Patients' clinicopathological characteristics by high and low value groups for each inflammatory biomarker are shown in Tables 2, 3 and 4, respectively. Cirrhosis was significantly associated with NLR and PLR, tumor size with NLR, and AJCC stage with LMR, but no other factors showed significant associations.

Overall survival

OS rates by each inflammatory biomarker are shown in Figure 3. Five-year OS rates were low-NLR: 83%, high-NLR: 42% ($P = 0.031$); high-LMR: 81%, low-LMR: 37% ($P < 0.01$); and low-PLR: 57%, high-PLR: 51% ($P = 0.84$).

Disease-free survival

The low LMR group had significantly worse disease-free survival (DFS) than the high LMR group. The high NLR group was likely to have worse DFS than the low NLR group, but there was not significant difference between them. Five-year DFS rates in low-NLR and high-NLR group were 56 % and 34 % respectively (P=0.23), and those in high-LMR and low-LMR group were 58 % and 30 % respectively (P=0.014).

For PLR, the high- and low-value groups did not significantly differ in DFS (Figure 4).

Comprehensive evaluation of inflammatory biomarkers

As NLR and LMR were significantly associated with OS in ICC patients, we designed an inflammation score (IS) system that allotted one point each to patients with high NLR, or low LMR, and two points for both, thus stratifying patients into three groups by scores 0, 1, or 2. Patients' 5-year OS significantly differed by their IS, at 0: 100%, 1: 61%, and 2: 32% (P = 0.011; Figure 5). Five-year DFS rates were not significantly different (P = 0.078; Figure 6).

Predictive factors for OS

Univariate and multivariate analyses are shown in Table 5 and 6. The cut-off values of absolute neutrophil and lymphocyte counts were determined using ROC curves that were based on mortality at 5 years after surgery. In univariate analysis, tumor size > 50 mm, AJCC classification stage III/IV, NLR, LMR, and IS were risk factors for shorter OS. In multivariate analysis, we did not include all inflammatory biomarkers in the same analysis because of the multicollinearity problem; we include single inflammatory biomarker in each analysis. For the same reason, we included only AJCC classification stage except for tumor size in the analyses. As prognostic factors, NLR, LMR, and IS were independent from AJCC classification stage.

Discussion

We found that NLR and LMR were significantly associated with long-term outcomes for MF-ICC patients. Moreover, the scoring system based on NLR and LMR was a possible prognostic factor for long-term outcome of MF-ICC patients. This implies a synergistic effect of NLR and LMR on long-term outcome, even though they

include a common factor (lymphocyte count), and are both indicators of inflammatory status in a host. Therefore, to identify high-risk patients, we should consider both of these inflammatory biomarkers. Although various assessment methods combine NLR and LMR, our simple method to calculate IS can discriminate among patients according to their prognoses, and is easy to use in clinical practice.

Tumor-infiltrating lymphocytes (TILs) have been suggested to be antitumor effector cells, and are associated with better long-term outcomes for HCC [23]. In our preliminary study, the ratio of lymphocytes among peripheral white blood cells was correlated with better prognosis (data not shown), and this might reflect the effect of TILs in the tumor microenvironment (TME). In the TME, neutrophils work as tumor-associated neutrophils (TANs) [24]. Furthermore, tumor-associated macrophages (TAMs) which originate from peripheral macrophages, are also important component of the TME [25]. TANs and TAMs have similar effect on tumor progression, such as tumor growth, extracellular matrix remodeling, angiogenesis, and immunosuppression, but they have some differences in signaling pathways [25]. These differences may have clinical implications, according to the results of our study. In our previous study, the

ratio of $CD3^+$ and $CD68^+$ cells in HCC sections (as shown by immunohistochemical analysis) were significantly associated with LMR values [26]. This is a rationale for the relationship between peripheral blood cell analysis and inflammation and immune status in the TME.

We found NLR to have no significant impact on DFS after surgery for MF-ICC. This negative result—especially considering the appearance of the Kaplan-Meier curve—may be due to the small sample size, which is this study's main limitation.

We could not show significance for PLR as a predictor for long-term outcome in MF-ICC patients, although a previous report showed PLR to be significantly associated with long-term outcomes in ICC [9]. Our study included only MF-ICC, which is reportedly relevant to hepatitis and liver cirrhosis [11, 12, 27, 28]. In our cohort, 56% had hepatitis (hepatitis B, hepatitis C, alcoholic hepatitis, and non-alcoholic steatohepatitis) and 13% had cirrhosis. Notably, the low PLR group tended to have high prevalence of hepatitis (low-PLR: 68%, high-PLR: 44% [P=0.10]) and a significantly high prevalence of cirrhosis (low-PLR: 28%, high-PLR: 0% [P=0.0036]). These may have affected the platelet counts and our results.

There were several limitations to this study. This was a retrospective analysis from a single center. Because we limited the cases to only MF-ICC without bile duct reconstruction, we had a small sample size. As cases with bile duct reconstruction may include those with hilar cholangiocarcinoma, we excluded them to eliminate possible unfavorable variability. We analyzed only MF type, but not PI type, IG type, and MF+PI type, because the latter three types are relatively rare and we were not able to collect adequate number of cases to conduct valid analyses. The intention of this limitation was to curb the heterogeneity due to different macroscopic types. Previous report revealed that NLR and LMR had significant impact on ICC, which had similar result as our study [10]. However, previous study did not consider the difference of macroscopic types. Our study shows that inflammatory biomarkers significantly affect MF-ICC, but may have different results for other macroscopic ICC types. As PI-ICC tended to have high PLR compared with MF-ICC, the usefulness of PLR may differ between MF-ICC and PI-ICC.

As for another limitation, some factors, such as cirrhosis in NLR and PLR, tumor size in NLR, and AJCC classification stage in LMR, were uneven between high

and low value groups, which reflects this study's retrospective design. Therefore, a larger-scale, multi-center prospective study would be necessary to strengthen the statistical validity and power.

We used cutoff values derived from ROC curves. Similar previous reports on cholangiocarcinoma that used ROC curves to determine cutoff values had values for NLR [8, 10, 29] and LMR [10, 30, 31] that were consistent with ours, but our study's PLR had a lower cutoff [9, 32]. A possible reason for the low PLR cutoff value was that the ROC AUC was small for PLR and the cutoff value for optimal sensitivity and specificity can be unstable. A larger-scale study is needed to decide the optimal cutoff value of each parameter and validate their wider applicability.

In conclusion, our study showed that the scoring system based on inflammatory biomarkers may predict long-term outcomes after surgery for ICC. Moreover, as its predictive value is independent of tumor stage, it may be helpful in identifying high-risk patients.

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Conflict of interest statement: Masafumi Ohira and other co-authors have no conflict of interest.

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Figure legends

Figure 1. A flowchart of patient inclusion and exclusion.

Figure 2. ROC curves to determine the cutoff values of each inflammatory biomarkers.

Figure 3. Comparison of overall survival rates between low-value and high-value groups for each inflammatory biomarker.

Figure 4. Comparison of disease-free survival rates between low-value and high-value groups for each inflammatory biomarker.

Figure 5. Comparison of overall survival rates for each inflammation score group.

Figure 6. Comparison of disease-free survival rates for each inflammation score group.

Table 1. Values of the inflammatory biomarkers by macroscopic types.

Inflammatory biomarkers	MF type (*n*=52)	MF+PI type (*n*=7)	PI type (*n*=4)	IG type (*n*=1)
NLR	2.57 (1.38)	2.59 (1.08)	2.74 (1.81)	4.73
LMR	4.56 (1.85)	4.20 (1.49)	5.33 (2.73)	1.4
PLR	145 (108)	140 (37)	163 (29)	218

Data are mean (standard deviation).

MF, mass forming; PI, periductal infiltrating; IG, intraductal growth; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

Table 2. Clinicopathological characteristics of the high-NLR and low-NLR patients

Factors	NLR < 1.93 (*n*=18)	NLR ≥ 1.93 (*n*=34)	*P*
Age, years	64 (44–82)	66 (39–82)	0.48
Sex			1
Male	14 (78%)	27 (79%)	
Female	4 (22%)	7 (21%)	
ICG-R15	13.6 (4.8–28.5)	8.6 (1.9–31.0)	0.061
Hepatitis	8 (44%)	21 (62%)	0.26
Cirrhosis	5 (28%)	2 (6%)	0.041
CEA, ng/ml	2.6 (0.7–41.8)	2.4 (0.4–21.3)	0.20
CA19-9, U/ml	25.4 (6.2–72)	37.0 (0.6–21100)	0.31
Tumor size, mm	30 (5-80)	48 (10-110)	0.015
LN metastasis	2 (11%)	5 (15%)	1
AJCC stage			1
I + II	12 (67%)	23 (68%)	
III + IV	6 (33%)	11 (32%)	
Adjuvant chemotherapy	7 (39%)	9 (26%)	0.37

Data are median (range) or *n* (%)

NLR, neutrophil-to-lymphocyte ratio; ICG-R15, indocyanine green retention 15 minutes after injection; LN, lymph node; AJCC, American Joint Committee on Cancer.

Table 3. Clinicopathological characteristics of high-LMR and low-LMR patients

Factors	LMR > 4.78 (*n*=20)	LMR ≤ 4.78 (*n*=32)	*P*
Age, years	61 (39–82)	67 (44–82)	0.18
Sex			0.082
Male	13 (65%)	28 (88%)	
Female	7 (35%)	4 (13%)	
ICG-R15	10.9 (2.3–28.5)	9.2 (1.9–31.0)	0.76
Hepatitis	9 (45%)	20 (63%)	0.26
Cirrhosis	3 (15%)	4 (13%)	1
CEA, ng/ml	2.35 (0.6–5.6)	2.55 (0.4–41.8)	0.15
CA19-9, U/ml	20.75 (3.3–293.7)	38.7 (0.6–21100)	0.28
Tumor size, mm	30 (16-94)	47 (5-110)	0.18
LN metastasis	1 (5%)	6 (19%)	0.23
AJCC stage			< 0.01
I + II	18 (90%)	17 (53%)	
III + IV	2 (10%)	15 (47%)	
Adjuvant chemotherapy	5 (25%)	11 (34%)	0.55

Data are shown as median (range) or *n* (%)

LMR, lymphocyte-to-monocyte ratio; ICG-R15, indocyanine green retention 15 minutes after injection; LN, lymph node; AJCC, American Joint Committee on Cancer.

Table 4. Clinicopathological characteristics of high-PLR and low-PLR patients

Factors	PLR < 98 (*n*=25)	PLR ≥ 98 (*n*=27)	*P*
Age, years	63 (44–82)	67 (39–82)	0.53
Sex			0.50
Male	21 (84%)	20 (74%)	
Female	4 (16%)	7 (26%)	
ICG-R15	11.5 (4.8–31.0)	9.2 (1.9–27.7)	0.097
Hepatitis	17 (68%)	12 (44%)	0.10
Cirrhosis	7 (28%)	0 (0%)	< 0.01
CEA, ng/ml	2.6 (0.7–41.8)	2.4 (0.4–8.8)	0.11
CA19-9, U/ml	31.3 (0.6–21100)	30.5 (3.3–3532)	0.51
Tumor size, mm	40 (12-100)	45 (5-110)	0.91
LN metastasis	3 (12%)	4 (15%)	1
AJCC stage			0.77
I + II	16 (64%)	19 (70%)	
III + IV	9 (36%)	8 (30%)	
Adjuvant chemotherapy	7 (28%)	9 (33%)	0.77

Data are median (range) or *n* (%)

PLR, platelet-to-lymphocyte ratio; ICG-R15, indocyanine green retention 15 minutes after injection; LN, lymph node; AJCC, American Joint Committee on Cancer.

Table 5. Univariate analyses of possible predictive factors for overall survival (OS).

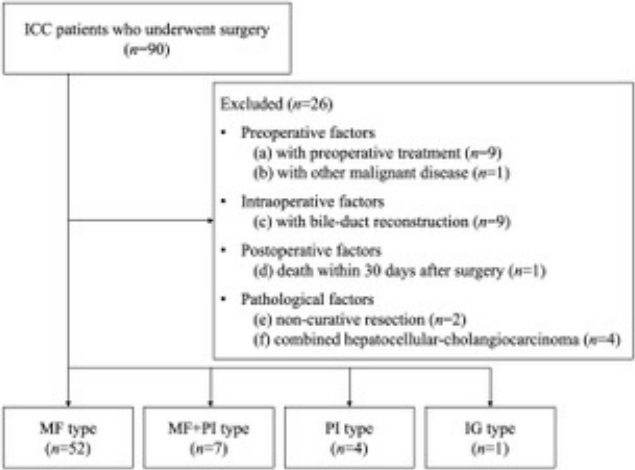
	Univariate analysis		
Factors	HR	95% CI	*P*
Age	1.025	0.981–1.071	0.27
Male sex	2.546	0.729–16.064	0.16
ICG-R15 >15 min	2.140	0.742–5.529	0.15
Hepatitis	1.732	0.684–4.934	0.25
Cirrhosis	1.808	0.418–5.499	0.38
CEA >5 ng/ml	1.956	0.557–5.402	0.27
CA19-9 >37 U/ml	1.669	0.672–4.204	0.27
Tumor size > 50mm	2.702	1.090–6.999	0.032
LN metastasis	2.421	0.685–6.750	0.15
AJCC stage III+IV	3.120	1.248–7.911	0.016
Adjuvant chemotherapy	0.693	0.224–1.813	0.47
Absolute neutrophil count ≥ 3737	1.760	0.672–5.453	0.26
Absolute lymphocyte count ≤ 1670	1.459	0.578-3.632	0.42
NLR ≥ 1.93	4.391	1.255–27.730	0.018
LMR ≤ 4.36	4.673	1.547–20.165	< 0.01
PLR ≥ 98	0.912	0.367–2.300	0.84
IS	3.320	1.542–9.121	< 0.01

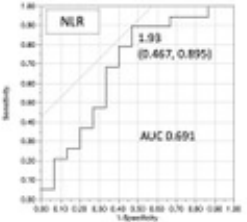
HR, Hazard ratio; 95% CI, 95% confidence interval; ICG-R15, indocyanine green retention 15 minutes after injection; LN, lymph node; AJCC, American Joint Committee on Cancer; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; IS, inflammation score.

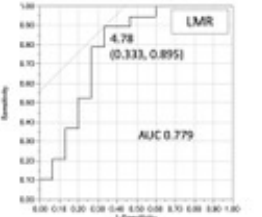
Table 6. Multivariate analyses of possible predictive factors for overall survival (OS).

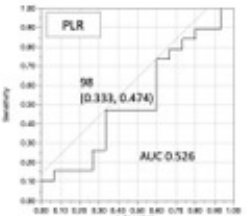
	Multivariate analysis		
	HR	95% CI	*P*
With NLR			
AJCC stage III+IV	2.867	1.148–7.263	0.025
NLR ≥ 1.93	4.007	1.144–25.324	0.028
With LMR			
AJCC stage III+IV	2.325	0.909–6.047	0.078
LMR ≤ 4.36	3.770	1.198–16.616	0.022
With IS			
AJCC stage III+IV	2.591	1.035–6.580	0.042
IS	3.277	1.454–9.317	< 0.01

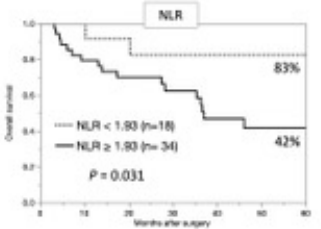
HR, Hazard ratio; 95% CI, 95% confidence interval; AJCC, American Joint Committee on Cancer; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; IS, inflammation score.

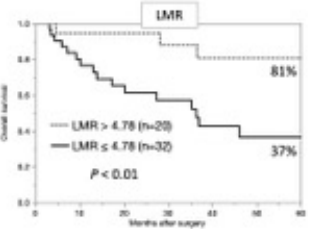

ICC patients who underwent surgery
(n=90)
Excluded (n=26)
• Preoperative factors
(a) with preoperative treatment (n=9)
(b) with other malignant disease (n=1)
• Intraoperative factors
(c) with bile-duct reconstruction (n=9)
• Postoperative factors
(d) death within 30 days after surgery (n=1)
• Pathological factors
(e) non-curative resection (n=2)
(f) combined hepatocellular-cholangiocarcinoma (n=4)
MF type
(n=52)
MF+PI type
(n=7)
PI type
(n=4)
IG type
(n=1)

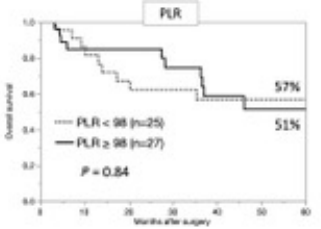

NLR
1.93
(0.467, 0.895)
AUC 0.691
Sensitivity
1-Specificity

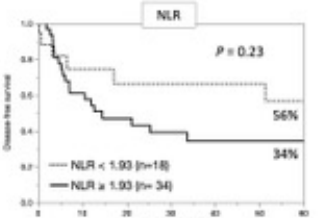

LMR
4.78
(0.333, 0.895)
AUC 0.779
Sensitivity
1-Specificity

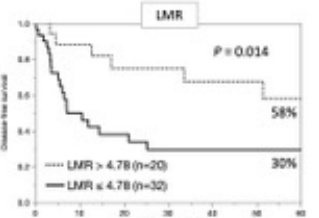

PLR
98
(0.333, 0.474)
AUC 0.526
Sensitivity
1-Specificity

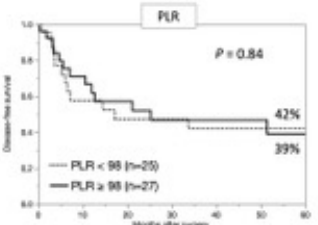

NLR
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
83%
42%
NLR < 1.93 (n=18)
NLR ≥ 1.93 (n= 34)
P = 0.031
0
10
20
30
40
50
60
Months after surgery

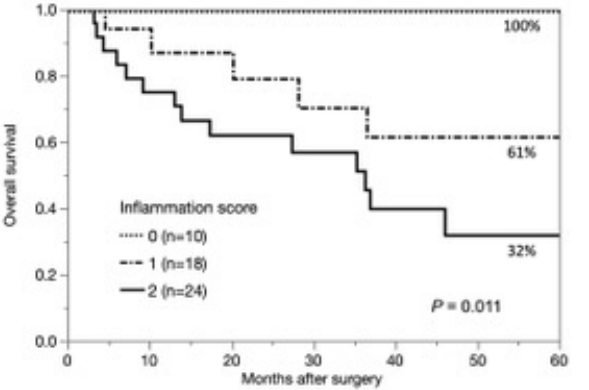

LMR
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
81%
37%
LMR > 4.78 (n=20)
LMR ≤ 4.78 (n=32)
P < 0.01
0
10
20
30
40
50
60
Months after surgery

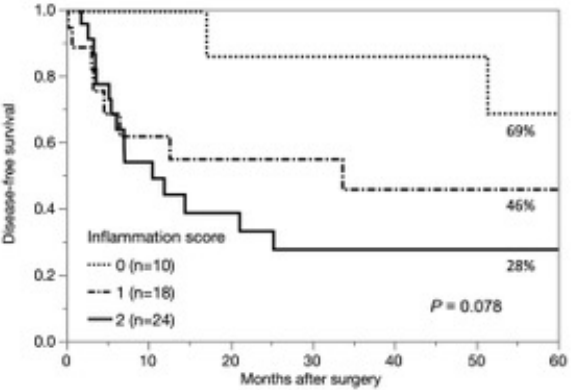

PLR
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
57%
51%
PLR < 98 (n=25)
PLR ≥ 98 (n=27)
P = 0.84
0
10
20
30
40
50
60
Months after surgery


NLR
P = 0.23
56%
34%
NLR < 1.93 (n=18)
NLR ≥ 1.93 (n= 34)
Disease-free survival
Months after surgery


LMR
P = 0.014
58%
30%
LMR > 4.78 (n=20)
LMR ≤ 4.78 (n=32)
Disease-free survival
Months after surgery


PLR
P = 0.84
42%
39%
PLR < 98 (n=25)
PLR ≥ 98 (n=27)
Disease-free survival
Months after surgery


1.0
0.8
0.6
0.4
0.2
0.0
Overall survival
0
10
20
30
40
50
60
Months after surgery
100%
61%
32%
Inflammation score
0 (n=10)
1 (n=18)
2 (n=24)
P = 0.011


1.0
0.8
0.6
0.4
0.2
0.0
Disease-free survival
0
10
20
30
40
50
60
Months after surgery
69%
46%
28%
Inflammation score
0 (n=10)
1 (n=18)
2 (n=24)
P = 0.078