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2 **Association of inflammatory biomarkers with long-term outcomes**

3 **after curative surgery for mass-forming intrahepatic**

4 **cholangiocarcinoma**

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3 **Key words:** Inflammatory biomarker, intrahepatic cholangiocarcinoma, long-term

4 outcome

5

6

1 **Abstract**

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

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

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

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

3 **Conclusions:** The IS system may predict long-term outcomes after surgery for MF-

4 ICC.

5

1 **Introduction**

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

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

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

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

4 **Methods**

5 *Patients*

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

1 *Surgical procedure and postoperative follow-up strategy*

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

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

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

6 *Inflammatory biomarkers*

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

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

1 *Outcomes and statistical analysis*

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

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

14 *Statistical analysis*

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

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

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

12 **Results**

13 *Sample size and inflammatory biomarker values classified by macroscopic types*

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

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

3 *Patient characteristics and inflammatory biomarker cutoff values*

4 We based our ROC curves on mortality at 5 years after surgery (Figure 2).

5 The cutoff values of inflammatory biomarkers were NLR: 1.93, LMR: 4.78, and PLR:

6 98. Patients' clinicopathological characteristics by high and low value groups for each

7 inflammatory biomarker are shown in Tables 2, 3 and 4, respectively. Cirrhosis was

8 significantly associated with NLR and PLR, tumor size with NLR, and AJCC stage with

9 LMR, but no other factors showed significant associations.

10

11 *Overall survival*

12 OS rates by each inflammatory biomarker are shown in Figure 3. Five-year

13 OS rates were low-NLR: 83%, high-NLR: 42% ($P = 0.031$); high-LMR: 81%, low-

14 LMR: 37% ($P < 0.01$); and low-PLR: 57%, high-PLR: 51% ($P = 0.84$).

1 *Disease-free survival*

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

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

9 *Comprehensive evaluation of inflammatory biomarkers*

10 As NLR and LMR were significantly associated with OS in ICC patients, we
11 designed an inflammation score (IS) system that allotted one point each to patients with
12 high NLR, or low LMR, and two points for both, thus stratifying patients into three
13 groups by scores 0, 1, or 2. Patients' 5-year OS significantly differed by their IS, at 0:
14 100%, 1: 61%, and 2: 32% ($P = 0.011$; Figure 5). Five-year DFS rates were not
15 significantly different ($P = 0.078$; Figure 6).

1 *Predictive factors for OS*

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

11

12 **Discussion**

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

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

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

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

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

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

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

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

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

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

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

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4

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

7

8

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4 Figure legends

5 Figure 1. A flowchart of patient inclusion and exclusion.

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

7 Figure 3. Comparison of overall survival rates between low-value and high-value

8 groups for each inflammatory biomarker.

9 Figure 4. Comparison of disease-free survival rates between low-value and high-value

10 groups for each inflammatory biomarker.

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

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

13

14

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

Inflammatory biomarkers	MF type (<i>n</i> =52)	MF+PI type (<i>n</i> =7)	PI type (<i>n</i> =4)	IG type (<i>n</i> =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 (<i>n</i> =18)	NLR ≥ 1.93 (<i>n</i> =34)	<i>P</i>
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)	<i>P</i>
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).

Factors	Univariate analysis		
	HR	95% CI	<i>P</i>
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	<i>P</i>
With NLR			
AJCC stage III+IV	2.867	1.148–7.263	0.025
NLR \geq 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 \leq 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.

111 patients after deconvolution surgery
(n=111)

Excluded (n=76)

- Respiratory failure
 - (a) with preoperative treatment (n=18)
 - (b) with other respiratory disease (n=1)
- Inadequate therapy
 - (c) with intracranial communication (n=6)
- Postoperative failure
 - (d) death within 30 days after surgery (n=1)
- Psychological factors
 - (e) pre-operative recovery (n=1)
 - (f) continued therapy with other drugs (n=1)

111 cases
(n=111)

111 cases
(n=111)

111 cases
(n=111)

111 cases
(n=111)









