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ORIGINAL ARTICLE

Preoperative lymphocyte/C-reactive protein ratio and its correlation with CD8$^+$ tumor-infiltrating lymphocytes as a predictor of prognosis after resection of intrahepatic cholangiocarcinoma

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Abstract

Purpose To clarify whether the preoperative lymphocyte/C-reactive protein (CRP) ratio (LCR) is a prognostic factor for patients with intrahepatic cholangiocarcinoma (IHCC), and investigate its mechanism via tumor-infiltrating lymphocytes.

Methods The subjects of this retrospective study were 42 patients who had undergone hepatectomy for IHCC. We divided the patients into low LCR and high LCR groups (cutoff value: 8780) and analyzed their overall survival (OS) and disease-free survival (DFS) with respect to LCR and other clinicopathological factors. We also investigated the levels of stromal tumor-infiltrating lymphocytes (TILs) and CD8$^+$ TILs in surgical specimens, and the relationship between LCR and TILs.

Results A low LCR was identified in 21 patients and was significantly correlated with older age, a high CRP-albumin ratio, and advanced disease stage, and was a prognostic factor for OS and DFS. Multivariate analysis revealed that a low LCR was an independent prognostic factor for worse OS (HR 10.40, $P=0.0077$). Although the LCR and levels of stromal TILs were not significantly related, LCR and levels of CD8$^+$ TILs were significantly related ($P=0.0297$).

Conclusion The preoperative LCR may predict the postsurgical prognosis of patients with IHCC and reflect the CD8$^+$ TILs.

Keywords Lymphocyte/C-reactive protein ratio · CD8$^+$ tumor-infiltrating lymphocytes · Stromal tumor-infiltrating lymphocytes · Intrahepatic cholangiocarcinoma

Abbreviations

ANC	Absolute neutrophil count
CAFs	Cancer associated fibroblasts
CRP	C-reactive protein
CAR	C-reactive protein–albumin ratio
DFS	Disease-free survival
H.E	Hematoxylin and eosin
IHCC	Intrahepatic cholangiocarcinoma
im	Intrahepatic metastasis
LCR	Lymphocyte–C-reactive protein ratio
LMR	Lymphocyte–monocyte ratio
LNM	Lymph-node metastasis
LPBC	Lymphocyte-predominant breast cancer
mGPS	Modified Glasgow Prognostic Score
NLR	Neutrophil–lymphocyte ratio
OS	Overall survival
PLR	Platelet–lymphocyte ratio
PNI	Prognostic nutrition index
PD-L1	Programmed death ligand 1
ROC	Receiver-operating characteristics
SII	Systemic immune inflammation index
TLC	Total lymphocyte count
TILs	Tumor-infiltrating lymphocytes
TMB	Tumor mutation burden
vp	Portal vein invasion
vv	Hepatic vein invasion

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Introduction

Various combinations of blood test indicators have been studied in the recent years, as prognostic indicators for cancer patients, including the neutrophil/lymphocyte ratio (NLR) [1, 2], the Modified Glasgow Prognostic Score (mGPS) [3, 4], and the Prognostic Nutrition Index (PNI) [5, 6], among others. These combinations can represent systemic inflammation, immune function, and/or nutritional status. The preoperative platelet/lymphocyte ratio (PLR), the systemic immune inflammation index (SII; platelets × NLR), the lymphocyte/monocyte ratio (LMR), and the C-reactive protein–albumin ratio (CAR), are all significantly associated with the outcome of patients who undergo curative resection of IHCC [7–10].

After Okugawa et al. described the lymphocyte/C-reactive protein (CRP) ratio (LCR), a new immune–nutritional index, as a strong prognostic factor for patients with colorectal cancer [11], the LCR was also found to indicate the prognosis of gastric cancer, pancreatic cancer, rectal cancer treated with chemoradiotherapy, and breast cancer [12–15]. Moreover, Lu et al. recently reported the LCR as a novel prognostic index in IHCC [16]. However, the mechanism of how the LCR reflects the prognosis of patients with IHCC is still unclear.

Although LCR and other immune–nutritional parameters represent the patient's systemic condition, tumor-infiltrating lymphocytes (TILs) may reflect local tumor immunity. Lymphocyte-predominant breast cancer (LPBC), which has high TIL levels, is associated with a good prognosis [17]. Lee et al. reported a significant correlation between the peripheral lymphocyte count and TILs in patients with cervical carcinoma [18], while Yoon et al. reported a significant relationship between TILs and the peripheral neutrophil count in patients with breast cancer [19]. $CD8^+$ lymphocytes are considered to be especially crucial among TILs. Zhu et al. reported a correlation between tumoral programmed death ligand 1 (PD-L1) expression and $CD8^+$ T-cell infiltration in IHCC patients [20], and Asahi et al. reported that numbers of $CD8^+$ T-cells in a tumor's outer border area were associated with the postsurgical outcomes of patients with IHCC [21].

In the present study, we investigated whether the preoperative LCR is a prognostic factor for patients with surgically treated IHCC, and clarified its mechanism via $CD8^+$ TILs.

Methods

Patients

The subjects of this retrospective study were 88 patients with IHCC diagnosed in Tokushima University Hospital between July, 2020 and May, 2005. Eight patients whose blood cell fraction was not measured before treatment were excluded from the analysis. We divided the patients into two groups: those with resectable IHCC ($n=45$) and those with unresectable IHCC ($n=35$). Three patients with resectable IHCC, whose follow-up was terminated for reasons other than death within 1 year after primary hepatectomy, were also excluded from the analysis. Finally, we enrolled 42 patients with resectable IHCC and 35 with unresectable IHCC. We collected the data on other clinicopathological features, such as age, gender, blood parameters, image findings, operative information, and pathological diagnosis, from the medical database. This study was approved by Tokushima University Hospital ethics committee with the approval of corresponding regulatory agencies, and all experiments were carried out in accordance with the approved guidelines (Tokushima Clinical Trial Management System Number; 3215). All the patients involved in this study signed informed consent forms and agreed to participate.

Treatment strategy for IHCC

Patients were treated according to a previously described strategy for resectable IHCC [22, 23]. Briefly, limited hepatectomy was performed without typical lymph node dissection or extrahepatic bile duct resection for peripheral tumors. For tumors in the perihilar region, or in the peripheral region with hilar infiltration, anatomical hepatectomy and regional lymph-node dissection were performed. Extrahepatic bile duct resection and reconstruction were performed if needed for surgical margins.

Preoperative immune parameters

Blood samples were taken prior to hepatectomy and the LCR was calculated by dividing the total lymphocyte count (TLC) (/μL) by the serum CRP (mg/dL) value. The optimal cutoff value for the LCR was calculated by using receiver operating characteristics (ROC) curves on mortality 5 years after surgery. Other immune–nutritional parameters, such as NLR, LMR, PLR, CAR, and PNI were classified into two groups by the median values.

Assessment of TILs

TILs were evaluated according to the standardized methodology proposed by the International TIL Working Group [24]. In summary, rather than counting the number

of lymphocytes, we assessed stromal TILs by calculating the ratio of TILs to stromal areas, excluding tumor cells, in hematoxylin and eosin (HE)-stained samples (Fig. 1a). We excluded the tumor cell areas (Fig. 1b) and then calculated the stromal and TILs areas (Fig. 1c) using the image analysis software ImageJ (National Institute of Health). Assessments were performed in areas within the borders of the invasive tumor, and tumor zones with crush artifacts, necrosis, regressive hyalinization were excluded. We scored all mononuclear cells, including lymphocytes, and plasma cells, but not polymorphonuclear leukocytes or fibroblasts. After a full assessment of average TILs in the tumor border area, we assessed the stromal TILs averaged among three selected high-power fields (400× magnification), not focused on hotspots. Thus, we evaluated TILs for 38 patients for whom HE slides were available, under the guidance of two expert pathologists, who were blinded to the clinical features of these patients.

Assessment of $CD8^+$ TILs

Anti-CD8 antibody (dilution 1:100, M7103; Dako) was used as the primary antibody. The immunohistochemistry procedures conducted in our department were those reported previously by Ishikawa et al. [25, 26]. Briefly, samples were formalin-fixed, paraffin-embedded, and cut into 5-μm-thick serial sections. Slides were dewaxed, deparaffinized in xylene, and rehydrated using a series of graded alcohol concentrations. Next, the slides were boiled with citrate buffer (pH 6.0) for 20 min by a microwave oven to activate antigen. To prevent nonspecific antigen binding, endogenous peroxidases were blocked with 0.3% hydrogen peroxide for 30 min, followed by incubation in 5% goat serum for 1 h. The slides were then incubated with anti-CD8 antibodies overnight at 4 °C. A secondary peroxidase-labeled polymer conjugated to goat antimouse immunoglobulins was applied for 1 h. The sections were developed with 3,3-diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin. Each slide was dehydrated using a graded series of alcohol concentrations and covered with a coverslip.

Selecting average regions within the tumor borders, we counted $CD8^+$ TILs in three high-power fields (400× magnification) per patient manually and calculated the mean number of $CD8^+$ TILs, for the 31 patients whose samples were available. Figure 2 shows representative cases of high (Fig. 2a) and low (Fig. 2b) $CD8^+$ TILs. These procedures were conducted under the guidance of two expert pathologists.

Statistical analysis

We used the unpaired Mann–Whitney U test or the χ^2 test to compare the clinicopathological variables between the two groups. The overall survival (OS) and disease-free survival (DFS) curves were created using the Kaplan–Meier method and the differences were analyzed by the log-rank

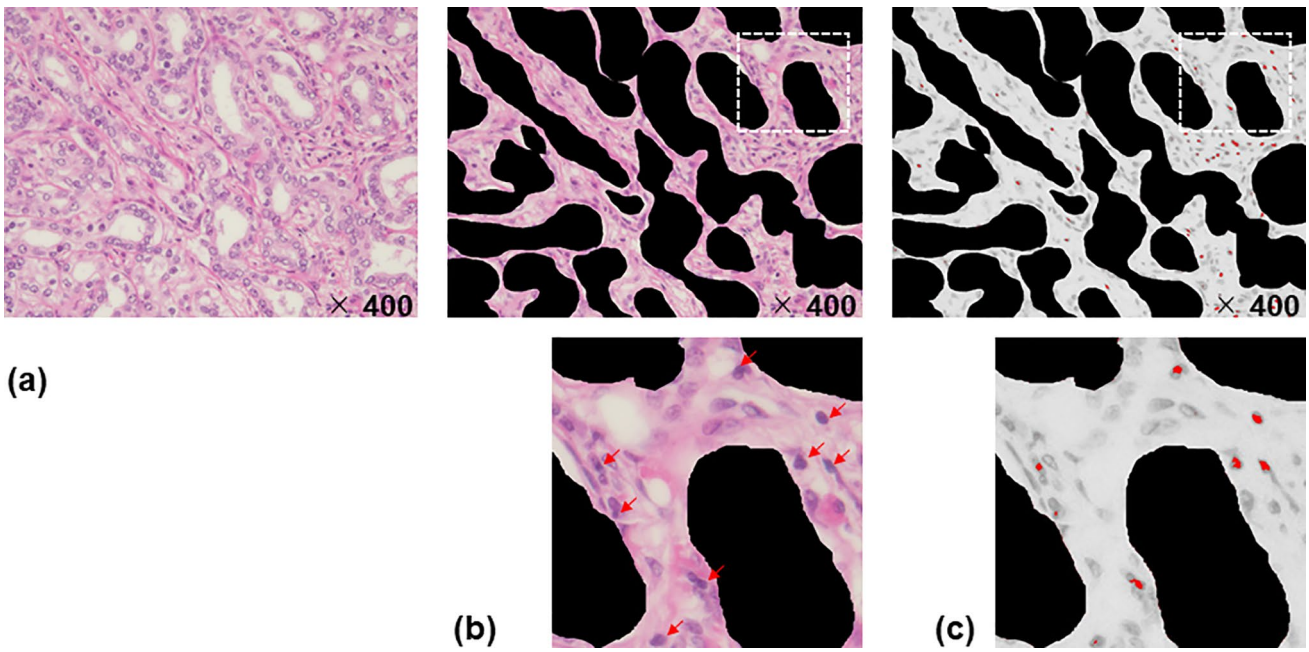

Fig. 1 Method of assessing tumor-infiltrating leukocytes (TILs) using ImageJ. **a** For each patient, we selected three average regions in high-power fields (×400 magnification). **b** We excluded the tumor cells (black). **c** We calculated TILs as the lymphocyte area (red)/stromal area (excluding black area) and averaged the score of the TILs in the three fields

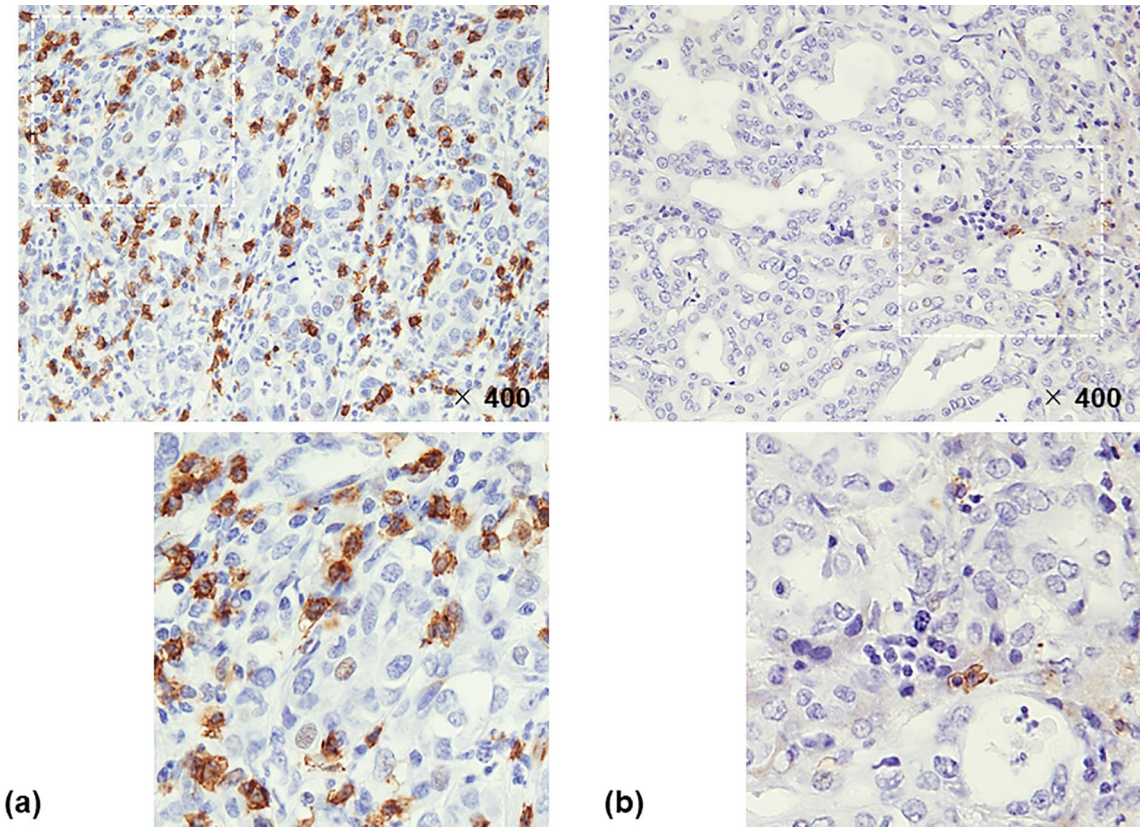

Fig. 2 Immunohistochemical staining for $CD8^+$ tumor-infiltrating lymphocytes (TILs). **a** Low $CD8^+$ TILs; **b** high $CD8^+$ TILs

test. Multivariate analysis was performed on factors identified by univariate analysis as having a significant difference. The Cox proportional hazard regression model was used for multivariate analysis. $P < 0.05$ was considered statistically significant.

Results

LCR in patients with resectable IHCC vs. in those with unresectable IHCC

Figure 3 shows the LCRs in patients with resectable IHCC vs. in those with unresectable IHCC. The median LCR was $12{,}245 \pm 9776$ in those with resectable IHCC and 2728 ± 5313 in those with unresectable IHCC, being significantly lower in those with unresectable IHCC ($P < 0.0001$).

Preoperative LCR can reflect postsurgical prognosis

The 5-year survival rate was 36.2% (Fig. 4a). The cutoff value of the LCR calculated by the ROC curve was 8780 (1 Specificity, Sensitivity: 0.133, 0.630; area under curve: 0.815; Fig. 4b). According to the cutoff value, patients were

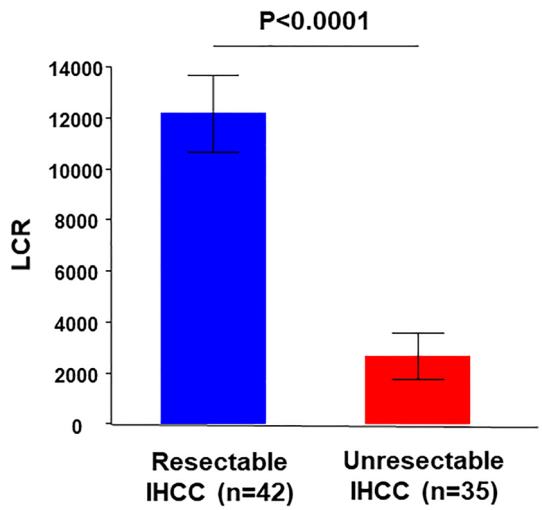

Fig. 3 The lymphocyte–C-reactive protein ratios (LCRs) in patients with resectable vs those with unresectable intrahepatic cholangiocarcinoma (IHCC). The LCR in patients with unresectable IHCC was significantly lower than that of those with resectable IHCC ($P < 0.0001$)

divided into a high LCR (LCR^{High}) group and a low LCR (LCR^{Low}) group. Table 1 summarized the clinicopathological features of two groups. Median values indicated that the LCR^{Low} group was significantly older, had high CAR, worse mGPS, and a longer operation time. The LCR^{Low} group also had more patients with curability C, intrahepatic and lymph-node metastasis (LMN), and more advanced disease than the LCR^{High} group. Other host, surgical, and tumor factors did not differ significantly between the two groups.

The 5-year OS rates after hepatectomy were significantly worse in the LCR^{Low} group (10.5%) than in the LCR^{High} group (58.3%; $P=0.0005$; Fig. 5a). In the univariate analysis, advanced age, low LCR, high CAR, worse mGPS, LNM, portal vein invasion (vp), advanced disease stage (III/IV), and high CA19-9 were associated with worse OS. In the multivariate analysis, low LCR, high CAR, LNM, and high CA19-9 were independent prognostic factors (Table 2). Furthermore, a low LCR was identified as the strongest of these prognostic factors (HR 10.40).

The 3-year DFS rates after hepatectomy were significantly worse in the LCR^{Low} group (0%) than in the LCR^{High} group (34.7%; Fig. 5b). Univariate analysis associated advanced age, low LCR, high CAR, bile duct resection, intrahepatic metastasis (im), LNM, vp, and advanced disease stage with poor prognosis. However, none of these factors were found to be independent prognostic factors in multivariate analysis (Table 3).

Preoperative LCR can reflect the number of local tumor $CD8^+$ TILs

The median percentages of TILs were 0.65% (interquartile range [IQR] 0.36–0.93) in the LCR^{High} group, and 0.55% (IQR 0.35–0.75) in the LCR^{Low} group. (Fig. 6a). The LCR and stromal TILs were not significantly associated. The median numbers of $CD8^+$ TILs were 25.4/slide (IQR 15.0–35.9) in the LCR^{High} group, and 12.3/slide (IQR 9.1–15.4) in the LCR^{Low} group (Fig. 6b). The number of $CD8^+$ TILs was related to the LCR ($P=0.0297$).

Discussion

The findings of the present study suggest that the preoperative LCR may be predictive of the postsurgical prognosis of patients who undergo resection for IHCC. Furthermore, as the LCR was associated with $CD8^+$ TILs, it reflects local tumor immune activity indirectly. To our knowledge, this is the first report to identify a correlation between a systemic immune marker and $CD8^+$ TILs in patients with IHCC.

The LCR was reported previously as a prognostic marker for colorectal, gastric, pancreatic, and breast cancers, and also for IHCC [11–16]. For patients who undergo curative resection of IHCC, the NLR, LMR, PLR, SII, and CAR have also been reported as significant prognostic factors [2, 7–10]. In this study, the LCR was the strongest prognostic factor for IHCC among these immune–nutritional parameters. The LCR is the combination of TLC and CRP. CRP is a well-established marker of inflammation. An elevated preoperative CRP level is reported widely to be associated

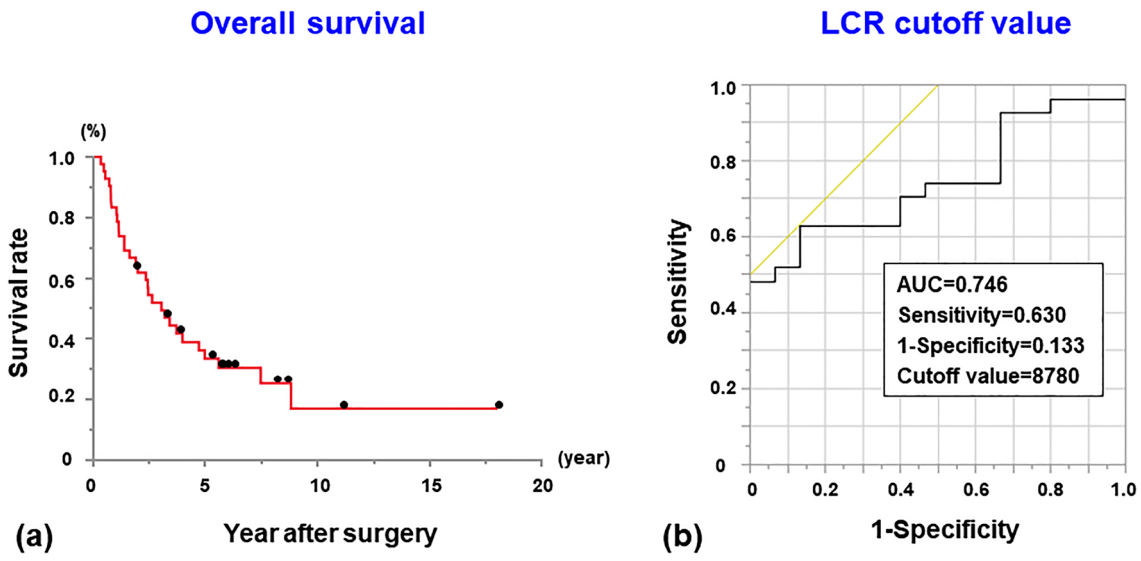

Fig. 4 Survival curve and ROC curve for the cutoff value of the lymphocyte–C-reactive protein ratio. **a** The 5-year overall survival of all patients was 36.2%. **b** ROC curve for the LCR (AUC = 0.746, sensitivity = 0.630, 1-specificity = 0.133, cutoff value = 8780)

Table 1 Clinicopathological factors in the high and low lymphocyte-CRP ratio groups

Variables	LCR > 8780 ($n = 23$)	LCR ≤ 8780 ($n = 19$)	P-value
Host factors			
Age (year)	67.7 ± 7.2	72.9 ± 6.8	0.0186
Gender (M/F)	16/7	15/4	0.4887
NLR	2.53 ± 1.19	3.38 ± 1.88	0.1327
LMR	5.37 ± 2.25	4.47 ± 1.69	0.3244
PLR	149 ± 76	161 ± 91	0.7617
CAR	0.023 ± 0.013	0.260 ± 0.469	< 0.0001
mGPS(0/1,2)	19/4	10/9	0.0353
PNI	47.1 ± 6.4	45.2 ± 5.0	0.4187
Frailty (−/+)	17/6	11/8	0.2731
HBV (−/+)	17/6	17/2	0.1908
HCV (−/+)	20/3	18/1	0.3801
Surgical factors			
Operation time (min)	342 ± 110	403 ± 66	0.0032
Blood loss (mL)	264 ± 189	406 ± 280	0.0669
Operation (Hr 0, S, 1/Hr 2, 3)	8/15	5/14	0.5532
Caudate lobectomy (−/+)	19/4	15/4	0.7640
Bile duct resection (−/+)	21/2	13/6	0.0574
Curability (A, B/C)	22/1	14/5	0.0376
Postoperative complications (−/+[a])	16/7	14/5	0.7683
Tumor factors			
Location (perihilar/hilar)	23/0	14/5	0.0031
Size (< 3 cm/≥ 3 cm)	6/17	5/14	0.9866
im (−/+)	21/2	12/7	0.0247
LNM (−/+)	18/5	12/7	0.2812
vp (−/+)	16/7	11/8	0.4324
vv (−/+)	21/2	16/3	0.4806
Stage (I, II/III, IV)	11/12	0/19	< 0.0001
CEA (< 10/≥ 10 ng/mL)	19/4	15/4	0.7640
CA19-9 (< 100/≥ 100 U/mL)	15/8	8/11	0.1329

CAR CRP-albumin ratio, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *im* intrahepatic metastasis, *LCR* lymphocyte-CRP ratio, *LMN* lymph node metastasis, *LMR* lymphocyte-monocyte ratio, *mGPS* modified Glasgow prognostic socre, *NLR* neutrophil-lymphocyte ratio, *PLR* platelet-lymphocyte ratio, *PNI* prognostic nutritional index, *vp* portal vein invasion, *vv* hepatic vein invasion.

[a]Complications of Clavien-Dindo ≥ IIIa were considered positive.

with the poor prognosis of patients with various cancers, including IHCC [27–29]. CRP is synthesized by hepatocytes in response to IL-6, TNF, and IL-1β, which can activate cancer cell proliferation. CRP may reflect systemic inflammation, and cancer cell proliferation and protection from apoptosis [30, 31]. TLC also reflects the host immune status and several papers have associated low TLC with poor prognosis [32, 33]. Thus, the LCR, which is the combination of CRP and TLC, may be a stronger prognostic factor than the other immune-nutritional parameters. A low LCR was also significantly associated with advanced age and longer operation time. The TLC can reflect the host's nutritional status, which can become compromised easily in elderly patients with malignancy. Because the low LCR group included many patients with advanced-stage disease ($P < 0.01$) and a higher frequency of bile duct resection ($P = 0.0574$), it was speculated that the addition of bile duct reconstruction and lymph-node dissection accounted for the longer operation times. However, the mechanism that links the LCR to long-term prognosis remains unclear. In the present study, we focused on the relationship between the LCR and TILs, which are indicators of local tumor immunity. TILs have attracted recent attention with the development of immune checkpoint inhibitors. Moreover, a relationship between TILs and peripheral blood cells, such as TLC and the absolute neutrophil count (ANC), has been reported [18, 19].

Interestingly, although this study found no significant relationship between the LCR and stromal TILs, the LCR

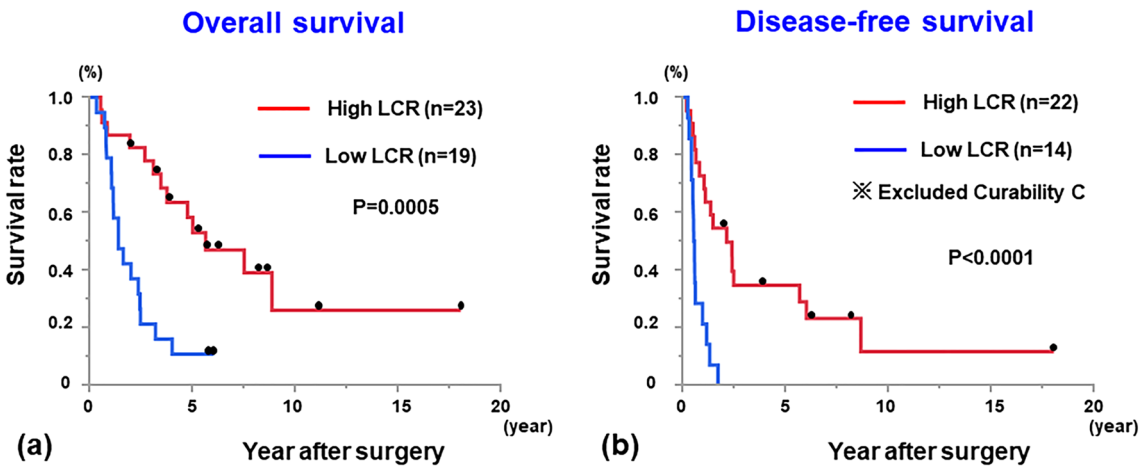

Fig. 5 Survival curves. **a** Overall survival rates of patients with a high or low lymphocyte/C-reactive protein ratio (LCR; $P = 0.0005$). **b** Disease-free survival rates of patients with a high or low LCR ($P < 0.0001$)

Table 2 Univariate and multivariate analyses for overall survival

Variables	5-year OS (%)	Univariate	Multivariate	
		P-value	HR (95% CI)	*P*-value
Host factors				
Age (< 75/≥ 75 year)	41.7/23.1	0.0343	1.77 (0.64–4.86)	0.2684
Gender (M/F)	35.0/41.6	0.7969		
LCR (> 8780/≤ 8780)	58.3/10.5	0.0005	10.40 (1.86–58.21)	0.0077
NLR (< 2.46/≥ 2.46)	53.3/21.2	0.1291		
LMR (> 4.81/≤ 4.81)	44.0/28.6	0.5952		
PLR (< 120/≥ 124)	40.4/32.1	0.9037		
CAR (< 0.0325/≤ 0.0325)	48.9/23.8	0.0391	6.65 (1.28–34.59)	0.0242
mGPS (0/1, 2)	47.5/9.2	0.0288	2.06 (0.86–4.94)	0.1061
PNI (> 46.9/≤ 46.9)	51.6/19.6	0.0585		
Frailty (−/+)	36.2/35.7	0.5032		
Operative factors				
Operation (Hr 0S1/Hr 23)	44.9/33.6	0.6723		
Caudate lobectomy (−/+)	37.2/30.0	0.8240		
Bile duct resection (−/+)	38.5/12.5	0.0587		
Curability (A, B/C)	39.5/16.7	0.2755		
Tumor factors				
Location (perihilar/hilar)	38.8/20.0	0.7256		
Size (< 3 cm/≥ 3 cm)	27.7/35.0	0.7940		
im (−/+)	40.3/22.2	0.1583		
LNM (−/+)	44.1/16.7	0.0147	3.26 (1.19–8.90)	0.0213
vp (−/+)	41.5/26.7	0.0339	2.92 (0.98–8.74)	0.0548
vv (−/+)	35.6/40.0	0.7881		
Stage (I, II/III, IV)	78.8/22.6	0.0007	1.64 (0.30–8.92)	0.5684
CEA (< 10/≥ 10 ng/mL)	35.9/37.5	0.7786		
CA19-9 (< 100/≥ 100 U/mL)	55.7/11.7	0.0003	4.12 (1.59–10.70)	0.0036

CAR CRP-albumin ratio, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *im* intrahepatic metastasis, *LCR* lymphocyte-CRP ratio, *LMN* lymph node metastasis, *LMR* lymphocyte-monocyte ratio, *mGPS* modified Glasgow prognostic socre, *NLR* neutrophil-lymphocyte ratio, *PLR* platelet-lymphocyte ratio, *PNI* prognostic nutritional index, *vp* portal vein invasion, *vv* hepatic vein invasion.

Table 3 Univariate and multivariate analyses for disease-free survival

Variables	3-year DFS (%)	Univariate	Multivariate	
		P-value	HR (95% CI)	*P*-value
Host factors				
Age (< 75/≥ 75 year)	28.3/0	0.0091	1.77 (0.47–6.73)	0.4013
Gender (M/F)	19.2/26.7	0.4908		
LCR (> 8780/≤ 8780)	34.7/0	< 0.0001	2.33 (0.53–10.17)	0.2622
NLR (< 2.46/≥ 2.46)	25.9/16.7	0.3387		
LMR (> 4.81/≤ 4.81)	25.3/17.7	0.4361		
PLR (< 124/≥ 124)	18.0/25.0	0.6399		
CAR (< 0.0325/≤ 0.0325)	33.0/0	0.0046	1.49 (0.31–7.23)	0.6226
mGPS (0/1, 2)	22.2/16.7	0.5804		
PNI (> 46.9/≤ 46.9)	22.2/20.0	0.6676		
Frail (−/+)	27.8/8.3	0.1880		
Operative factors				
Operation (Hr 0S1/Hr 23)	43.6/12.0	0.1349		
Caudate lobectomy (−/+)	20.0/33.3	0.3498		
Bile duct resection (−/+)	25.5/0	0.0092	2.97 (0.89–9.92)	0.0774
Tumor factors				
Location (perihilar/hilar)	23.9/0	0.2190		
Size (< 3 cm/≥ 3 cm)	26.7/19.2	0.2114		
im (−/+)	26.3/0	0.0009	2.91 (0.96–8.85)	0.0594
LNM (−/+)	29.4/0	0.0066	1.63 (0.64–4.20)	0.3089
vp (−/+)	28.7/7.7	0.0291	1.33 (0.42–4.19)	0.6281
vv (−/+)	20.6/25.0	0.4986		
Stage (I, II/III, IV)	60.6/4.0	< 0.0001	2.24 (0.51–9.75)	0.2835
CEA (< 10/≥ 10 ng/mL)	22.0/16.7	0.4083		
CA19-9 (< 100/≥ 100 U/mL)	27.3/14.3	0.2157		

CAR CRP-albumin ratio, *CA19-9* carbohydrate antigen 19-9, *CEA* carcinoembryonic antigen, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *im* intrahepatic metastasis, *LCR* lymphocyte-CRP ratio, *LMN* lymph node metastasis, *LMR* lymphocyte-monocyte ratio, *mGPS* modified Glasgow prognostic socre, *NLR* neutrophil-lymphocyte ratio, *PLR* platelet-lymphocyte ratio, *PNI* prognostic nutritional index, *vp* portal vein invasion, *vv* hepatic vein invasion.

and $CD8^+$ TILs, specifically, were associated. The TIL volume is reportedly correlated with tumor mutation burden (TMB) [34]. TMB is the number of gene mutations that cancer cells have and is associated with a predicted immunotherapy response [35]. In breast cancer, which is reported to have relatively high TMB and TILs, patients who have more than 50% TILs are often defined as having LPBC with < 10% TILs considered minimal [24]. In the present study, the median TIL percentage in all patients was 0.43% (IQR 0.26–0.77), which was much lower than that for breast cancer. Biliary cancer is also reported to have relatively low TMB [36]. Thus, TILs in IHCC were expected to be low and our result was consistent with that. A low TIL percentage in IHCC may be why the LCR and TILs did not correlate. Furthermore, the TIL evaluation method included all mononuclear cells, not only lymphocytes. As IHCC seems to be associated with low TILs, it is possible that cells other than lymphocytes were affected.

TILs include various types of lymphocytes, such as $CD3^+$, $CD4^+$, and $CD8^+$ cells, with $CD8^+$ T cells considered especially important. Tumor infiltration by $CD8^+$ T cells has been strongly associated with the survival of cancer patients [21, 24]. There are only a few reports showing the relationship between the systemic immune marker and $CD8^+$ TILs. CRP is produced in the liver in response to inflammatory cytokines from tumor microenvironment. These inflammatory cytokines have an immunosuppressive effect on the tumor microenvironment. Kato T, et al. reported that cancer-associated fibroblasts (CAFs) reduced $CD8^+$ TILs through the secretion of high levels of IL6 [37]. Mitchell et al. found that neutrophils also reduced $CD8^+$ TILs by producing inflammatory cytokines [38]. Kinoshita et al. reported that high expression of IL-38, a member of the IL-1 family, was also associated with decreased $CD8^+$ TILs [39]. Yoshida et al. reported that CRP inhibited the proliferation, activation, and function

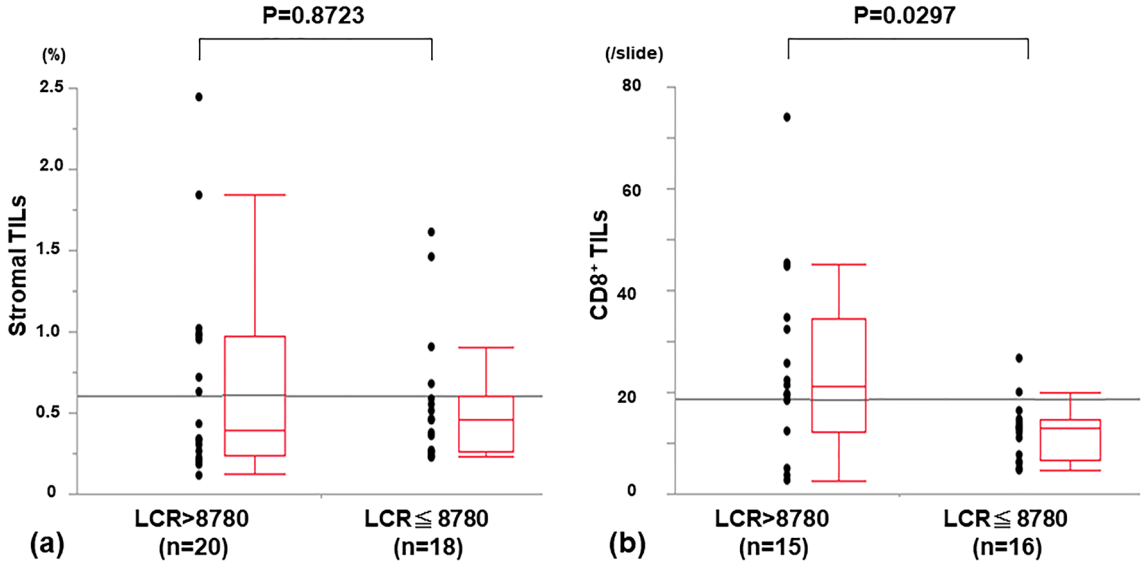

Fig. 6 Association between the LCR and TILs. **a** No significant association was found between the lymphocyte/C-reactive protein ratio (LCR) and tumor-infiltrating leukocytes (TILs) in all types. **b** The lymphocyte/C-reactive protein ratio (LCR) was significantly related to $CD8^+$ TILs ($P = 0.0297$)

of $CD8^+$ T cells in patients with melanoma [40]. CRP may reflect the total amount of inflammatory cytokines produced not only by cancer cells, but also by CAFs, neutrophils, and other cells constituting the tumor microenvironment. On the other hand, peripheral blood lymphocytes may play an important role in TIL formation and tumor immunity. It has been reported that $CD8^+$ TILs and TLC showed positive correlation in breast cancer and esophageal cancer [41, 42]. Recently, Okadome, et al. reported that high $CD8^+$ TILs were correlated positively with PNI, which includes TLC [43]. Furthermore, Shirasawa M, et al. reported that the NLR was inversely correlated with $CD8^+$ TILs in lung cancer [44]. There are several reports on the relationship between immune–nutritional parameters, using TLC and CD8 TILs. Thus, $CD8^+$ TILs may be more strongly associated with LCR, which is a robust prognostic factor. LCR can reflect not only the inflammatory response and immune–nutritional status, but also local tumor immunity via $CD8^+$ TILs.

$CD8^+$ TILs have been reported as the predictor of response to chemotherapy or immune check point therapy [24]. Thus, preoperative LCR may predict the response to adjuvant therapy. IHCC has a high recurrence rate, not only for local recurrence, but also for intrahepatic recurrence or distant metastasis. Reduced surgery may be better tolerated by patients with a low LCR to maintain general condition. Furthermore, in patients with a low LCR, nutrition and exercise therapy before and after surgery may contribute to a better prognosis. Although there is no highly effective treatment other than surgery for IHCC, an immune checkpoint inhibitor is expected to be a "game changer" [45]. In the context of immune checkpoint therapy, the LCR could be a highly useful biomarker via $CD8^+$ TILs.

This study had several limitations. First, it was a retrospective analysis from a single center, with a relatively small study cohort. Second, although lymphocytes come in various types, such as $CD3^+$ or $CD4^+$ lymphocytes, we assessed only $CD8^+$ T cells as the most representative type for tumor immunity. A larger study, and an assessment of non-$CD8^+$ lymphocytes are issues needing future study.

In conclusion, the LCR can predict the prognosis of patients undergoing surgery for IHCC, reflective of the $CD8^+$ TILs. The LCR may also be a predictor of the response to immune checkpoint inhibitor.

Declarations

Conflict of interest We have no conflicts of interest to declare.

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