# C-reactive protein/albumin ratio and modified Glasgow prognostic score are associated with prognosis and infiltration of Foxp3+ or CD3+ lymphocytes in colorectal liver metastasis

**Hiroki Kanno** ( kanno_hiroki@med.kurume-u.ac.jp )
Kurume University School of Medicine

**Toru Hisaka**
Kurume University School of Medicine

**Jun Akiba**
Kurume University School of Medicine

**Kazuaki Hashimoto**
Kurume University School of Medicine

**Fumihiko Fujita**
Kurume University School of Medicine

**Yoshito Akagi**
Kurume University School of Medicine

**Research Article**

**Keywords:** colorectal liver metastasis, inflammatory index, tumor-infiltrating lymphocytes

**Posted Date:** March 29th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1464310/v1

# Abstract

**Background**: Inflammatory indices and tumor-infiltrating lymphocytes (TILs) have prognostic value in many cancers. This study aimed to assess the prognostic value of inflammatory indices and evaluate their correlation with survival and the presence of TILs in colorectal liver metastasis (CRLM).

**Methods**: Medical records of 117 patients who underwent hepatectomy for CRLM were retrospectively reviewed. We calculated inflammatory indices such as neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, C-reactive protein/albumin ratio (CAR), and modified Glasgow prognostic score (mGPS) and evaluated their relationship with survival rates and immunohistochemical results of tumor-infiltrating CD3+, CD8+, and Foxp3+ lymphocytes.

**Results:** Patients with low CAR values and low mGPS had significantly better overall survival in the log-rank test (p=0.0246 and p=0.0121, respectively). In multivariate analysis using the Cox proportional hazard model, CAR (hazard ratio [HR], 0.5717; 95% confidence interval [CI], 0.3288–0.9939; p=0.0475) and mGPS (HR, 0.4000; 95% CI, 0.1934–0.8270; p=0.0134) were independent prognostic factors. Additionally, Foxp3+ lymphocytes were more common in samples of patients with low CAR (p=0.0409), and the number of CD3+ TILs was significantly higher in patients with low mGPS (p=0.0152).

**Conclusions:** CAR and mGPS are simple, inexpensive, and objective markers associated with survival of patients with CRLM. Moreover, they can predict the presence of Foxp3+ and CD3+ lymphocytes in the invasive margin of the tumor.


# Background

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide [1]. Approximately 33% of patients with CRC develop metastasis during their clinical course, which worsens their survival. Liver is the most common site of metastasis in CRC, and liver resection remains the only potential curative treatment for colorectal liver metastasis (CRLM), with a 5-year survival rate of approximately 44–49.6% [2,3]. Nevertheless, the recurrence rate after hepatectomy for CRLM is approximately 62% [4]. Therefore, it is necessary to identify effective biomarkers to predict the risk of CRLM recurrence and the chances of survival.

Systemic inflammation and cancer are closely related. Cancer-related inflammation can affect tumor progression, which in turn induces systemic inflammation [5,6]. In several cancers, including CRLM, inflammatory indices such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), C-reactive protein/albumin ratio (CAR), and modified Glasgow prognostic score (mGPS)—which assigns categorical values between 0 and 2 depending on the combination of serum levels of C-reactive protein and albumin— have been proven to have good prognostic value [7-11]. Thus, systemic inflammation is a key factor in tumorigenesis. However, the detailed mechanism remains unclear.

The host tumor microenvironment plays a pivotal role in cancer progression. Some studies have reported that the presence of tumor-infiltrating lymphocytes (TILs) influences the long-term outcome of many cancers [12-14]. In CRC, a high number of CD3+ and/or CD8+ TILs is associated with better prognosis, whereas a high number of Foxp3+ TILs can be indicative of either better or worse prognosis, depending on the primary tumor site and other accompanying factors [15,16]. Although several studies have investigated the relationship between inflammatory indices and the presence of TILs [17,18], there have been no reports on this topic in CRLM.

Therefore, the present study aimed to explore the prognostic value of inflammatory indices, such as NLR, PLR, CAR, and mGPS, and their correlation with the presence of TILs in CRLM.

## Methods

### *Patients*

The present study was approved by the Research Ethics Committee of Kurume University, Kurume, Japan (approval number: 21228) and conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived because of the retrospective nature of the study. The medical records of 202 Japanese patients who underwent hepatectomy for CRLM at Kurume University between January 2006 and December 2019 were retrospectively analyzed. Among them, 85 cases were excluded because of the following reasons: 34 cases had undergone repeated hepatectomy for CRLM, and 33 cases had metastases in other sites, including the lungs, lymph nodes, and/or peritoneum. Curative resection was not achieved in 5 cases. In 13 cases, pathological evaluation was not performed due to missing samples. Therefore, the data of pathological confirmed 117 patients were reviewed.

### *Data collection*

Clinicopathological data were obtained from the patients' medical records. Blood samples were collected during the week prior to surgery. For neoadjuvant chemotherapy (NAC), we selected oxaliplatin- or irinotecan-based regimens with/without therapeutic monoclonal antibodies. Postoperative follow-up was conducted as follows: levels of tumor markers such as carcinoembryonic antigen (CEA) and/or carbohydrate antigen 19-9 were measured every three months after surgery. Computed tomography was performed every 3–6 months after surgery. Recurrence-free survival (RFS) was defined as the time from surgery to recurrence or death. Overall survival (OS) was defined as the time from surgery to death.

### *Calculation and cutoff value of each inflammatory index*

Each inflammatory index was calculated as follows: NLR, ratio between peripheral blood neutrophil count and peripheral blood lymphocyte count; PLR, ratio between peripheral blood platelet count and peripheral blood lymphocyte count; CAR, ratio between serum C-reactive protein (CRP) concentration and

serum albumin concentration. The mGPS was calculated using both CRP and albumin concentrations. The corresponding cutoff values were <1.0 mg/dL for CRP and ≥3.5 g/dL for albumin. If both CRP and serum albumin concentrations were within reference values, the mGPS was set to 0. If either one of them was out of reference, the mGPS was set to 1. If both CRP and albumin concentrations were out of reference values, mGPS was set to 2. Median values were used as cutoff values for NLR, PLR, and CAR. For each index, we categorized patients in two groups ("low" or "high") depending on their results with respect to the cutoff. For mGPS, "low" corresponds to mGPS = 0 and "high" corresponds to mGPS = 1 or 2.

## *Immunohistochemistry*

Formalin-fixed paraffin-embedded tissue slides were cut at 4 μm, examined on a coated glass slide, and labeled with the following antibodies using the Bond-III autostainer (Leica Microsystems, Newcastle, UK): anti-CD3 antibody (×300, clone LN10, Leica Microsystems, Newcastle, UK), anti-CD8 antibody (×200, clone 4B11, Leica Microsystems), and anti-Foxp3 antibody (×100, clone 236A/E7, Abcam, Cambridge, MA, USA). Briefly, samples for anti-CD3, -CD8, and -Foxp3 antibodies were heat-treated using epitope retrieval solution 2 (pH 9.0) at 99°C for 15, 15, and 30 min, respectively, and then incubated with each antibody for 30 min at room temperature. This automated system uses a Refine detection system (Leica Microsystems, Newcastle, UK) with a horseradish peroxidase-conjugated polymer as the secondary reagent and 3,3’ diaminobenzidine as the chromogen.

## *Evaluation of tumor-infiltrating lymphocytes*

The total number of TILs (expressing CD3, CD8, and Foxp3) in the tumor center (TC) and invasive margin (IM) was counted separately. To reduce the effect of tumor heterogeneity, three well-stained spots at ×400 magnification were evaluated in all samples, and the average of the three measurements was used for analyses. Representative immunohistochemistry images are shown in Figure 1.

## *Statistical analyses*

Clinicopathological characteristics of the patients were compared using the chi-square test for categorical variables and Mann–Whitney U test for continuous variables. Survival curves were created using the Kaplan–Meier method and compared using the log-rank test. A Cox proportional hazards model was used for univariate and multivariate analyses, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. The variables showing significant associations with RFS or OS in the univariate analysis were included in the multivariate analysis. All statistical analyses were performed using the JMP Pro version 15 (SAS Institute, Cary, NC, USA). A p value <0.05 was considered statistically significant.

# Results

## Patient characteristics

Clinicopathological characteristics of patients with low or high NLR, PLR, CAR, and mGPS values are summarized in Table 1. There were more male patients in the high NLR group (p=0.0166). Liver tumor diameter was significantly larger in the high PLR, CAR, and mGPS groups (p=0.0011, p<0.0001, and p=0.0108, respectively). The percentage of patients receiving NAC was significantly higher in the high CAR group (p=0.0449).

## Comparison of RFS and OS between low and high NLR, PLR, CAR, and mGPS groups

The RFS and OS curves of the low and high NLR, PLR, CAR, and mGPS groups are shown in Figure 2. RFS curves were not significantly different between groups. However, the low CAR and low mGPS groups were associated with significantly better OS (p=0.0246 and p=0.0121, respectively). In NLR and PLR, there were no significant differences between the two groups.

## Univariate and multivariate analyses for RFS and OS

The results of univariate and multivariate analyses for RFS and OS are shown in Tables 2 and 3. Univariate analysis showed that NAC (no vs. yes; HR, 0.4191; 95% CI, 0.2668–0.6585; p=0.0002), onset (metachronous vs. synchronous; HR, 0.3681; 95% CI, 0.2308–0.5873; p<0.0001), tumor location (bilober vs. hemilober; HR, 1.6491; 95% CI, 1.0518–2.5856; p=0.0292), and tumor number (solitary vs. multiple; HR, 0.5973; 95% CI, 0.3796–0.9401; p=0.0260) were prognostic factors for RFS. In multivariate analysis, onset (metachronous vs. synchronous; HR, 0.4656; 95% CI, 0.2776–0.7810; p=0.0038) was an independent prognostic factor. Univariate analysis demonstrated that NAC (no vs. yes; HR, 0.5780; 95% CI, 0.3384–0.9871; p=0.0447), onset (metachronous vs. synchronous; HR, 0.5014; 95% CI, 0.2887–0.8710; p=0.0143), tumor location (bilober vs. hemilober; HR, 1.8626; 95% CI, 1.0853–3.1966; p=0.0240), CAR (<0.030 vs. 0.030≤; HR, 0.5386; 95% CI, 0.3113–0.9318; p=0.0269), and mGPS (0 vs. 1/2; HR, 0.4095; 95% CI, 0.1992–0.8418; p=0.0152) were the prognostic factors for OS. On multivariate analysis, CAR (<0.030 vs. 0.030≤; HR, 0.5717; 95% CI, 0.3288–0.9939; p=0.0475) and mGPS (0 vs. 1/2; HR, 0.4000; 95% CI, 0.1934–0.8270; p=0.0134) were the independent prognostic factors.

## Relationship between each inflammatory index and the presence of TILs

We evaluated the relationship between each inflammatory index and the presence of TILs. Foxp3+, a marker of regulatory T-cells, was expressed more intensely in the IM of patients with low CAR (p=0.0409), and the number of CD3+TILs in the IM was significantly higher in patients of the low mGPS group

(p=0.0152) (Figure 3). Differences in the number of other TILs between groups were not significant (Additional files 1-3).

# Discussion

In this study, we investigated the utility of inflammatory indices, such as NLR, PLR, CAR, and mGPS, in terms of the long-term outcomes of CRLM after hepatectomy. Clinicopathological features differed between groups in terms of sex, tumor diameter, and percentage of patients who underwent NAC. The low CAR and mGPS groups showed significantly better OS in the log-rank test, and low CAR and low mGPS were independent prognostic factors of better OS in univariate and multivariate analyses. Moreover, the low CAR group was related to high infiltration of Foxp3+ lymphocytes, and a low mGPS was associated with a high number of CD3+ TILs.

Both CAR and mGPS comprise CRP and albumin levels. CRP is an acute phase protein secreted by hepatocytes in response to acute inflammatory stimuli through the production of interleukin-1 (IL-1) and interleukin-6 (IL-6). Some cytokines, including IL-1 and IL-6, are released during cancer progression [19]. Thus, CRP levels may reflect tumor activation. Serum albumin level is an indicator of immune nutritional status. Hypoalbuminemia induces an impaired immune response, which in turn, promotes cancer growth. Additionally, decreased albumin levels demonstrate an increased inflammatory status with elevated levels of cytokines, such as tumor necrosis factor-alpha, IL-1, and IL-6, which may contribute to cancer progression [20]. Therefore, high CAR or high mGPS may reflect impaired tumor immunity and suggest tumor progression.

The present study showed that Foxp3+ and CD3+ lymphocytes were more commonly found in samples of the low CAR and low mGPS groups, respectively. Nakayama et al. revealed an association between CRP and TILs in renal cell carcinoma: higher CRP levels indicated a stronger infiltration of CD8+, Foxp3+, and CD163+ TILs [18]. Martin et al. showed that lower CAR levels were related to the presence of CD8+ TILs [17]. Foxp3+ lymphocytes have been reported to suppress anti-tumor immunity, which worsens survival [21,22]. However, many studies have revealed that the presence of Foxp3+ lymphocytes is associated with favorable prognosis in primary colorectal cancer [12,15,16,23]. Ladoire et al. described that a possible reason for this discrepancy may be the characteristics of the gut microbiota. They suggest that Foxp3+ lymphocytes may prevent bacteria-driven inflammation and carcinogenesis [24]. However, there are generally no microbiomes in the liver. Alternatively, high Foxp3 expression has been associated with better OS in hepatocellular carcinoma patients based on its capacity to inhibit the expression of the oncogenic protein c-Myc and induce apoptosis in tumor cells [25]; thus, the association of high Foxp3 expression with good prognosis in CRLM needs further investigation.

The International Study Group of Pancreatic Surgery recommends preoperative determination of the mGPS in patients with borderline resectable pancreatic cancer [26]. The present study demonstrated that both CAR levels and mGPS have prognostic value for OS in CRLM. While CAR consists of continuous

variables such as CRP and serum albumin levels, mGPS consists of categorical variables. In the present study, only 11.1% of patients were categorized as having mGSP 1 or 2. Other reports on CRLM also show that only 11–14% of cases are categorized as mGPS 1 or 2 [27,28]. Thus, we believe that CAR may sufficiently represent the systemic inflammatory status and have a good predictive value for survival in patients with CRLM.

In CRLM, multidisciplinary therapy, including NAC, is pivotal; therefore, effective predictors of chemotherapy or immune checkpoint inhibitors responses are warranted to prolong survival. Some researchers have reported that the response to immune checkpoint inhibitors differs according to the number of TILs [29,30]. However, TILs can only be evaluated using surgical specimens, and biopsy samples cannot overcome sampling errors and tumor heterogeneity. Our study indicates that some inflammatory indices are associated with the presence of TILs. This means that inflammatory indices, rather than TILs' status, may be good predictors of response to neoadjuvant therapy.

The present study had some limitations. First, there is a potential risk of selection bias due to the single-center retrospective design. Second, NAC was administered to some patients, which might have influenced the systemic inflammatory status and the presence of TILs. Third, other immune cells, such as CD4+, CD163+, PD-1+, and PD-L1+ TILs, were not evaluated in the present study, which could be helpful for the comprehensive understanding of the relationship between systemic inflammation and the tumor microenvironment.

In conclusion, CAR and mGPS are simple, inexpensive, and objective assessment tools for evaluating the inflammatory status of patients with CRLM and predicting survival. In addition, they can indicate the presence of certain types of TILs in the IM of the tumor.

# Abbreviations

CAR: C-reactive protein to albumin ratio, CEA: carcinoembryonic antigen, CI: confidence interval, CRC: colorectal cancer, CRLM: colorectal liver metastasis, CRP: C-reactive protein, HR: hazard ratio, IM: invasive margin, mGPS: modified Glasgow prognostic score, NAC: neoadjuvant chemotherapy, NLR: neutrophil to lymphocyte ratio, OS: overall survival, PLR: platelet to lymphocyte ratio, TC: tumor center, TILs: tumor-infiltrating lymphocytes, RFS: recurrence-free survival

# Declarations

### *Ethics approval and consent to participate*

The present study was approved by the Research Ethics Committee of Kurume University, Kurume, Japan (approval number: 21228) and conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the Research Ethics Committee of Kurume University because of the retrospective nature of the study.

*Consent for publication*:

None declared.

*Availability of data and materials*:

All the data generated or analyzed during this study are included in this published article [and its supplementary information files].

**Competing interests**:

None declared.

**Funding**:

None declared.

**Authors' contributions:**

Conception and design: H. Kanno, Development of methodology: H. Kanno, J. Akiba.

Data collection: H. Kanno, F. Fujita, Analysis and interpretation of data: H. Kanno, Writing, review and/or revision of the manuscript: H. Kanno, T. Hisaka, Study supervision: T. Hisaka, Y. Akagi.

**Acknowledgements**:

We thank Akihiko Kawahara for his technical assistance, and Editage for editing the manuscript (https://www.editage.com).

# References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin. 2021;71:7-33.
2. Scherman P, Syk I, Holmberg E, Naredi P, Rizell M. Influence of primary tumour and patient factors on survival in patients undergoing curative resection and treatment for liver metastases from colorectal cancer. BJS Open. 2020;4:118-32.
3. Väyrynen V, Wirta EV, Seppälä T, Sihvo E, Mecklin JP, Vasala K, et al. Incidence and management of patients with colorectal cancer and synchronous and metachronous colorectal metastases: a population-based study. BJS Open. 2020;4:685-92.
4. Butte JM, Gönen M, Allen PJ, Peter Kingham T, Sofocleous CT, DeMatteo RP, et al. Recurrence After partial hepatectomy for metastatic colorectal cancer: potentially curative role of salvage repeat resection. Ann Surg Oncol. 2015;22:2761-71.
5. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436-44.

6. McMillan DC. Cancer and systemic inflammation: stage the tumour and stage the host. Br J Cancer. 2013;109:529.
7. Ma W, Zhang P, Qi J, Gu L, Zang M, Yao H, et al. Prognostic value of platelet to lymphocyte ratio in hepatocellular carcinoma: a meta-analysis. Sci Rep. 2016;6:35378.
8. Conci S, Campagnaro T, Danese E, Lombardo E, Isa G, Vitali A, et al. Role of inflammatory and immune-nutritional prognostic markers in patients undergoing surgical resection for biliary tract cancers. Cancers (Basel). 2021;13.
9. Sakamoto Y, Mima K, Imai K, Miyamoto Y, Tokunaga R, Akiyama T, et al. Preoperative C-reactive protein-to-albumin ratio and clinical outcomes after resection of colorectal liver metastases. Surg Oncol. 2020;35:243-8.
10. Haruki K, Shiba H, Horiuchi T, Sakamoto T, Gocho T, Fujiwara Y, et al. Impact of the C-reactive protein to albumin ratio on long-term outcomes after hepatic resection for colorectal liver metastases. Am J Surg. 2017;214:752-6.
11. Deng Y, Zhao Y, Qin J, Huang X, Wu R, Zhou C, et al. Prognostic value of the C-reactive protein/albumin ratio and systemic immune-inflammation index for patients With colorectal liver metastasis undergoing curative resection. Pathol Oncol Res. 2021;27:633480.
12. Kanno H, Miyoshi H, Yoshida N, Sudo T, Nakashima K, Takeuchi M, et al. Differences in the immunosurveillance pattern associated with DNA mismatch repair status between right-sided and left-sided colorectal cancer. Cancer Sci. 2020;111:3032-44.
13. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960-4.
14. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005;353:2654-66.
15. Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol. 2009;27:186-92.
16. deLeeuw RJ, Kost SE, Kakal JA, Nelson BH. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: a critical review of the literature. Clin Cancer Res. 2012;18:3022-9.
17. Martin D, Rödel F, Balermpas P, Winkelmann R, Fokas E, Rödel C. C-reactive protein-to-albumin ratio as prognostic marker for anal squamous cell carcinoma treated with chemoradiotherapy. Front Oncol. 2019;9:1200.
18. Nakayama T, Saito K, Kumagai J, Nakajima Y, Kijima T, Yoshida S, et al. Higher serum C-reactive protein level represents the immunosuppressive tumor microenvironment in patients with clear cell renal cell carcinoma. Clin Genitourin Cancer. 2018;16:e1151-e8.
19. Saito K, Kihara K. C-reactive protein as a biomarker for urological cancers. Nat Rev Urol. 2011;8:659-66.

20. Kim S, Mcclave SA, Martindale RG, Miller KR, Hurt RT. Hypoalbuminemia and clinical outcomes: what is the mechanism behind the relationship? Am Surg. 2017;83:1220-7.
21. Kiryu S, Ito Z, Suka M, Bito T, Kan S, Uchiyama K, et al. Prognostic value of immune factors in the tumor microenvironment of patients with pancreatic ductal adenocarcinoma. BMC Cancer. 2021;21:1197.
22. Zheng X, Jin W, Wang S, Ding H. Progression on the roles and mechanisms of tumor-infiltrating T lymphocytes in patients with hepatocellular carcinoma. Front Immunol. 2021;12:729705.
23. Berntsson J, Svensson MC, Leandersson K, Nodin B, Micke P, Larsson AH, et al. The clinical impact of tumour-infiltrating lymphocytes in colorectal cancer differs by anatomical subsite: A cohort study. Int J Cancer. 2017;141:1654-66.
24. Ladoire S, Martin F, Ghiringhelli F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. Cancer Immunol Immunother. 2011;60:909-18.
25. Gong Z, Jia H, Yu J, Liu Y, Ren J, Yang S, et al. Nuclear FOXP3 inhibits tumor growth and induced apoptosis in hepatocellular carcinoma by targeting c-Myc. Oncogenesis. 2020;9:97.
26. Bockhorn M, Uzunoglu FG, Adham M, Imrie C, Milicevic M, Sandberg AA, et al. Borderline resectable pancreatic cancer: a consensus statement by the International Study Group of Pancreatic Surgery (ISGPS). Surgery. 2014;155:977-88.
27. Nakagawa K, Tanaka K, Nojiri K, Kumamoto T, Takeda K, Ueda M, et al. The modified Glasgow prognostic score as a predictor of survival after hepatectomy for colorectal liver metastases. Ann Surg Oncol. 2014;21:1711-8.
28. Okimoto S, Kobayashi T, Tashiro H, Kuroda S, Ishiyama K, Ide K, et al. Significance of the Glasgow Prognostic Score for patients with colorectal liver metastasis. Int J Surg. 2017;42:209-14.
29. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019;19:133-50.
30. Plesca I, Tunger A, Müller L, Wehner R, Lai X, Grimm MO, et al. Characteristics of tumor-infiltrating lymphocytes prior to and During immune checkpoint inhibitor therapy. Front Immunol. 2020;11:364.

# Tables

**Table 1**

Clinicopathological characteristics of the patients according to the level of inflammatory indices

| | Low NLR | High NLR | | Low PLR | High PLR | | Low CAR | High CAR | | mGPS: 0 | mGPS: 1/2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N=58 | N=59 | P-value | N=58 | N=59 | P-value | N=58 | N=59 | P-value | N=104 | N=13 | P-value |
| Sex | | | 0.0166* | | | 0.4006 | | | 0.2198 | | | 0.2919 |
| Male | 31 | 44 | | 35 | 40 | | 34 | 41 | | 65 | 10 | |
| Female | 27 | 15 | | 23 | 19 | | 24 | 18 | | 39 | 3 | |
| Age (median, range), years | 66 (38-81) | 66 (33-83) | 0.5948 | 65.5 (33-81) | 67 (41-83) | 0.7537 | 68 (33-83) | 64 (38-83) | 0.1960 | 65.5 (33-83) | 70 (45-83) | 0.1460 |
| BMI (median, IQR) | 21.9 (20.1-23.5) | 22.1 (20.3-25.6) | 0.4600 | 23.2 (20.5-24.8) | 21.2 (19.6-24.2) | 0.0963 | 22.5 (20-23.9) | 21.8 (20.4-25.5) | 0.6391 | 21.8 (20.1-24) | 23.6 (21-25.8) | 0.2038 |
| Primary tumor location | | | 0.9548 | | | 0.4391 | | | 0.7202 | | | 0.3550 |
| Right sided | 16 | 16 | | 14 | 18 | | 15 | 17 | | 27 | 5 | |
| Left sided | 42 | 43 | | 44 | 41 | | 43 | 42 | | 77 | 8 | |
| NAC | | | 0.5211 | | | 0.1621 | | | 0.0449* | | | 0.4568 |
| No | 33 | 37 | | 31 | 39 | | 40 | 30 | | 61 | 9 | |
| Yes | 25 | 22 | | 27 | 20 | | 18 | 29 | | 43 | 4 | |
| Preoperative CEA (median, IQR) | 8.8 3.7-19.7) | 12.5 (3.9-45.2) | 0.0890 | 8.8 (3.7-19.8) | 12.3 (5.1-47.9) | 0.0546 | 8.8 (.6-28.3) | 12.2 (4.5-34) | 0.2626 | 9.4 (3.9-26.4) | 1.3 (3.7-174.9) | 0.1918 |
| Onset | | | 0.7783 | | | 0.6464 | | | 0.6464 | | | 0.8958 |
| Metachronous | 27 | 29 | | 29 | 27 | | 29 | 27 | | 50 | 6 | |
| Synchronous | 31 | 30 | | 29 | 32 | | 29 | 32 | | 54 | 7 | |
| Metastatic tumor location | | | 0.0738 | | | 0.3058 | | | 0.6191 | | | 1.0000 |
| Bilobar | 27 | 18 | | 25 | 20 | | 21 | 24 | | 40 | 5 | |
| Hemilobar | 31 | 41 | | 33 | 39 | | 37 | 35 | | 64 | 8 | |
| Liver tumor diameter (median, IQR) | 25 (17.8-39.3) | 25 (20-53) | 0.1968 | 24 (15-34.3) | 33 (22-60) | 0.0011* | 23 (16.8-32.3) | 38 (22-61) | <.0001* | 25 (20-40) | 60 (21.5-87.5) | 0.0108* |
| Tumor number | | | 0.4078 | | | 0.6480 | | | 0.4078 | | | 0.5558 |
| Solitary | 29 | 25 | | 28 | 26 | | 29 | 25 | | 47 | 7 | |
| Multiple | 29 | 34 | | 30 | 33 | | 29 | 34 | | 57 | 6 | |

BMI: body mass index, CAR: C-reactive protein/albumin ratio, CEA: carcinoembryonic antigen, IQR: interquartile range, mGPS: modified Glasgow prognostic score, NAC: neoadjuvant chemotherapy, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio

## Table 2

Univariate and multivariate analyses of recurrence-free survival

| | Univariate | | | | Multivariate | | |
|---|---|---|---|---|---|---|---|
| | 95% CI | HR | p-value | | 95% CI | HR | p-value |
| Sex ( male vs. female) | 0.7419-1.9042 | 1.1189 | 0.4725 | | | | |
| Age (<70 vs. ≥70 years) | 0.8058-2.0697 | 1.2914 | 0.2879 | | | | |
| Primary tumor location (Right vs. Left) | 0.7097-1.8874 | 1.1574 | 0.5580 | | | | |
| NAC (No vs. Yes) | 0.2668-0.6585 | 0.4191 | 0.0002* | | 0.3663-1.0498 | 0.6201 | 0.0752 |
| Preoperative CEA (<5 vs. ≥5) | 0.5179-1.4161 | 0.8564 | 0.5457 | | | | |
| Onset (Metachronous vs. Synchronous) | 0.2308-0.5873 | 0.3681 | <.0001* | | 0.2776-0.7810 | 0.4656 | 0.0038* |
| Tumor location ( Bilobar vs. Hemilobar ) | 1.0518-2.5856 | 1.6491 | 0.0292* | | 0.6720-2.1618 | 1.2053 | 0.5310 |
| Tumor diameter (<50 vs. ≥50) | 0.4175-1.2264 | 0.7156 | 0.2234 | | | | |
| Tumor number ( Solitary vs. Multiple ) | 0.3796-0.9401 | 0.5973 | 0.0260* | | 0.5345-1.8025 | 0.9815 | 0.9521 |
| NLR (<2.03 vs. ≥2.03) | 0.5812-1.4138 | 0.9065 | 0.6651 | | | | |
| PLR (<134 vs. ≥134) | 0.6019-1.4672 | 0.9397 | 0.7844 | | | | |
| CAR (<0.030 vs. ≥0.030) | 0.4996-1.2149 | 0.7791 | 0.2708 | | | | |
| mGPS (0 vs. 1/2) | 0.4342-1.7453 | 0.8706 | 0.6961 | | | | |

CAR: C-reactive protein/albumin ratio, CEA: carcinoembryonic antigen, CI: confidence interval, HR: hazard ratio, mGPS: modified Glasgow prognostic score, NAC: neoadjuvant chemotherapy, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio

## Table 3

Univariate and multivariate analyses of overall survival

| | Univariate | | | | Multivariate[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 95% CI | HR | p-value | | 95% CI | HR | p-value | | 95% CI | HR | p-value |
| Sex ( male vs. female) | 0.5744-1.7583 | 1.0049 | 0.9862 | | | | | | | | |
| Age (<70 vs. ≥70 years) | 0.6042-1.9074 | 1.0735 | 0.8089 | | | | | | | | |
| Primary tumor location (Right vs. Left) | 0.6096-2.0102 | 1.1070 | 0.7384 | | | | | | | | |
| NAC (No vs. Yes) | 0.3384-0.9871 | 0.5780 | 0.0447* | | 0.4703-1.5853 | 0.8635 | 0.6358 | | 0.4355-1.4607 | 0.7976 | 0.4639 |
| Preoperative CEA (<5 vs. ≥5) | 0.3442-1.2055 | 0.6442 | 0.1690 | | | | | | | | |
| Onset (Metachronous vs. Synchronous) | 0.2887-0.8710 | 0.5014 | 0.0143* | | 0.3254-1.1478 | 0.6111 | 0.1257 | | 0.3208-1.0860 | 0.5902 | 0.0901 |
| Tumor location ( Bilobar vs. Hemilobar ) | 1.0853-3.1966 | 1.8626 | 0.0240* | | 0.8446-2.6897 | 1.5072 | 0.1650 | | 0.8480-2.6596 | 1.5017 | 0.1632 |
| Tumor diameter (<50 vs. ≥50) | 0.3323-1.2071 | 0.6333 | 0.1651 | | | | | | | | |
| Tumor number ( Solitary vs. Multiple ) | 0.3648-1.0950 | 0.6321 | 0.1018 | | | | | | | | |
| NLR (<2.03 vs. ≥2.03) | 0.5023-1.4621 | 0.8570 | 0.5713 | | | | | | | | |
| PLR (<134 vs. ≥134) | 0.4571-1.3407 | 0.7828 | 0.3724 | | | | | | | | |
| CAR (<0.030 vs. ≥0.030) | 0.3113-0.9318 | 0.5386 | 0.0269* | | 0.3288-0.9939 | 0.5717 | 0.0475* | | — | | |
| mGPS (0 vs. 1/2) | 0.1992-0.8418 | 0.4095 | 0.0152* | | — | | | | 0.1934-0.8270 | 0.4000 | 0.0134* |

[a]Adjusted for the following variables: NAC, Onset, Tumor location

CAR: C-reactive protein/albumin ratio, CEA: carcinoembryonic antigen, CI: confidence interval, HR: hazard ratio, mGPS: modified Glasgow prognostic score, NAC: neoadjuvant chemotherapy, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio

# Figures

**Figure 1**

Representative immunohistochemical images of CD3+, CD8+ and Foxp3+ lymphocytes in the tumor center (TC) and invasive margin (IM).

**Figure 2**

Kaplan–Meier curves of RFS and OS according to the levels of inflammation indices.

CAR, C-reactive protein/albumin ratio; mGPS, modified Glasgow prognostic factor; NLR, neutrophil/lymphocyte ratio; OS, overall survival; PLR, platelet/lymphocyte ratio; RFS, recurrence-free survival

**Figure 3**

Relationship between the level of inflammatory indices and the presence of tumor-infiltrating lymphocytes.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportingfile1.tif
- Supportingfile2.tif
- Supportingfile3.tif
- Supportingfile1.tif
- Supportingfile2.tif
- Supportingfile3.tif