Research Article

Prognostic impact of hepatic steatosis evaluated by CT on immunotherapy for gastric cancer; associations with sarcopenia, systemic inflammation, and hormones

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Short Title:

Hepatic steatosis in immunotherapy of gastric cancer

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Abstract

Introduction: Immune checkpoint inhibitors (ICIs) are expected to improve the prognosis of gastric cancer (GC). On the other hand, hepatic steatosis has been reported to be associated with cancer cachexia, and expected to be a cancer biomarker. The purpose of this study is to evaluate prognostic impact of hepatic steatosis in ICIs therapy for GC.

Methods: Unresectable or recurrent GC treated with ICIs were investigated. Using unenhanced CT, liver to spleen CT attenuation ratio (LSR) was calculated as a parameter of hepatic steatosis. LSR was compared with the presence of sarcopenia, inflammatory markers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR). These parameters were also compared with disease specific survival (DSS) and progression free survival (PFS). Associations of LSR with insulin-like growth factor 1 (IGF-1) and growth hormone were also evaluated.

Results: A total of seventy patients were investigated. LSR of sarcopenia patients was significantly lower than that of non-sarcopenia ones (P=0.02). LSR showed significant negative correlations with NLR, PLR, and MLR (P=0.003, 0.03, 0.01, respectively). Lower LSR was significantly associated with higher level of serum IGF-1 (P=0.03). In univariate analysis, LSR was significantly correlated with DSS and PFS (both P<0.0001), and multivariate analysis demonstrated that LSR was the independent prognostic factor for both DSS and PFS (both P=0.01). ROC analysis demonstrated that LSR>1.263 was a good predictive marker for favorable DSS (>5.3 months) with AUC of 0.80.

Conclusion: Hepatic steatosis can be a promising prognostic biomarker for ICIs therapy of GC, associated with sarcopenia and the elevation of inflammatory markers. Out data suggested that GC with steatohepatitis might be less responsive to ICIs therapy.

Introduction

Immunotherapy with immune checkpoint inhibitors (ICIs) such as programmed death-1 (PD-1) monoclonal antibodies can contribute greatly to the improvement of treatment outcomes for several malignancies. Since the recent phase III data from ATTRACTION-2 study [1], nivolumab, which is one of PD-1 monoclonal antibodies, has been recommended for unresectable advanced or recurrent gastric or gastro-esophageal junction cancer refractory to, or intolerant of, two or more previous regimens of chemotherapy. Pembrolizumab, another PD-1 monoclonal antibody, was also approved for advanced gastric or gastro-esophageal junction cancer patients after failure of first-line chemotherapy [2]. According to the ATTRACTION-2 study, the 1-year overall survival (OS) rate of Nivolumab treatment was 26.2% [1]. Similarly, the KEYNOTE-059 study reported that the 1-year OS rate of pembrolizumab therapy was 23.4% [2]. Both studies reported that anti-PD-1 antibody treatment was obviously better than placebo, but about 75% of patients died within 1-year. Besides, considering the far advanced status of cancer at the time of initiation of these anti-PD-1 antibody treatments in gastric cancer patients, biomarkers for the treatment of these ICIs are highly desirable.

On the other hand, cancer cachexia has been reported to be associated with poor prognosis in various types of advanced cancer [3]. In the research of cachexia, the majority of previous studies have focused on the loss of skeletal muscle and adipose tissue [4]. However, recent studies reported the link between the steatohepatitis and cancer cachexia, and it was suggested that alterations in hepatic lipid metabolism might play an important role in the manifestation of cancer cachexia [5-8]. Therefore, this cancer-associated steatohepatitis (CASH) must have a clinical impact on the management of cancer treatment. However, to our knowledge, there has been no clinical study on the relationship between hepatic steatosis and prognosis in ICIs therapy of any types of cancers. Because of a possible association between steatohepatitis and cancer cachexia, quantitative evaluation of hepatic steatosis must be a prognostic biomarker for ICIs therapy of cancers, as recent two papers have reported a relationship of the skeletal muscle loss due to cachexia with survival in patients non–small cell lung cancer patients treated with nivolumab [9,10]. In addition, it was reported that the presence of excess fat in the liver of patients with non-alcoholic steatohepatitis (NASH) was obviously associated with the immune system from the study of hepatocellular carcinoma (HCC) [11]. In fact, a recent report suggested that ICIs were ineffective against NASH-related HCC [12]. Therefore, we speculate that the severity of hepatic steatosis can be a biomarker for ICIs therapy in advanced gastric cancer. Unenhanced CT is a simple and objective method capable of accurately quantifying hepatic steatosis. It has been reported that the calculation of liver to spleen CT attenuation ratio (LSR) on unenhanced CT can be a reliable marker for steatohepatitis [13,14]. Therefore, this study investigates whether hepatic steatosis measured by CT can predict survival of gastric cancer patients treated with ICIs, compared with markers of cancer cachexia such as sarcopenia and systemic inflammatory markers.

Materials and Methods

Patient population

This retrospective study protocol was approved by the Clinical Research Ethics Committee at Chiba University Hospita. Written informed consent for participation was not required because of the retrospective nature of this study. We retrospectively identified pathologically proven unresectable advanced or recurrent gastric adenocarcinoma patients treated with Nivolumab or Pembrolizumab in our institute from October 2018 to December 2021. The diagnosis of unresectable or recurrent gastric adenocarcinoma was made by our well-experienced surgical oncology team for gastric cancer (K.H., M.K., H.S., Y.M., Y.K., R.O.; board certified surgeons in gastroenterology with more than 10 years of experience in the treatment of gastric cancer), based on clinical data including blood test, pathology, and imaging examinations such as CT, MRI, or FDG-PET. All patients received at least first-line chemotherapy before ICIs therapy.

Treatment and follow-up

The treatment schedule and the dose modification schema of ICIs therapy with the use of Nivolumab or Pembrolizumab have been detailed previously [1,15]. Nivolumab was administered intravenously at a dose of 240 mg every 2 weeks, while Pembrolizumab was administered intravenously at a dose of 200 mg every 3 weeks. These treatments were continued until disease progression, unacceptable toxicity, or patient refusal. Tumor responses were assessed by board certified radiologists with CT every 2-4 cycles of the chemotherapy. The tumor markers including carcinoembryonic antigen and CA 19-9, were measured every month. After these therapies, any additional treatment occurred at the discretion of the treating physician, but basically followed Japanese gastric cancer treatment guidelines [16].

Imaging acquisition and assessment of hepatic steatosis

All patients were examined on a 320/80-section multi-detector row CT (MDCT) scanner (Aquilion ONE VISION / Aquilion PRIME; Canon Medical Systems, Otawara, Japan) within one month before or after the date of initiation of ICIs therapy. If a patient received multiple CT scans during this period, the CT on the closest date to the start date of the treatment was used in this analysis. The following CT parameters were used for acquisition of volume data: 120 kVp; 150-200 mA; 0.5-second rotation time; field of view, 360; matrix, 512; pixel size, 0.7mm x 0.7mm; 5 mm reconstructed slice thickness. The volumetric CT dose index was approximately 8-12 mGy. Regarding the measurement of hepatic steatosis, the mean CT attenuation value of the liver and that of the spleen were obtained using a

single oval region-of-interest (ROI) of about 200 mm2, taking care to avoid vessels, calcifications, focal fatty change, and masses, if present (Fig. 1). Thereafter, liver to spleen ratio (LSR; liver attenuation / spleen attenuation) was calculated as the quantitative marker for hepatic steatosis [13,14,17].

Assessment of sarcopenia from skeletal muscle

Psoas muscle index (PMI) at the third lumbar vertebra level was calculated as a marker for sarcopenia on CT image. The following equation was applied to calculation of PMI; PMI (cm2 / m2) = cross-sectional area of both psoas muscle (cm2) / height2 (m2). These parameters were measured using unenhanced CT images. Generally, the PMI cut-off values for sarcopenia are 6.36 cm2/m2 in male patients and 3.92 cm2/m2 in female patients, based on a previous report, but this criteria is for the healthy young Asian adults [18]. However, our patients were relatively older and had undergone one or more chemotherapy regimens, and most patients categorized as sarcopenia based on this proposed definition. Therefore, this study defined low PMI (sarcopenia) as < 3.9 cm2/ m2 for male patients and < 2.5 cm2/m2 for female patients according to the median values of PMIs of men and women in this cohort. This analysis was performed by a single observer (K.H., with 18 years of experience in CT interpretation).

Systemic inflammatory markers related to cachexia

Systemic inflammatory markers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) were calculated as following; NLR was calculated by dividing absolute neutrophil count by lymphocyte count, PLR was by dividing thrombocyte count by lymphocyte count, and LMR was by dividing monocyte count by lymphocyte count measured in peripheral blood on the day of the first administration of ICIs. Serum C-reactive protein (CRP) was also measured in peripheral blood on the day of the first administration of ICIs.

Measurement of insulin-like growth factor 1 and growth hormone

Serum level of insulin-like growth factor 1 (IGF-1) was analyzed and measured with an immunoradiometric assay (IRMA) at a single laboratory (SRL, Tokyo, Japan). Serum growth hormone (GH) level was analyzed and measured by electrochemiluminescence immunoassay (ECLIA). These blood tests for hormones were required for all patients who received ICIs therapy in our institute, in

case of endocrine adverse events due to ICIs, and were acquired within one month before the date of initiation of ICIs therapy.

Statistical analysis

Statistical analyses were carried out using the JMP 13.0 (SAS Institute, Inc., Cary, NC, USA), and for all comparisons, $P < 0.05$ was considered to indicate a statistically significant difference. Mann-Whitney U test was used for comparison of two independent data sets. Relationships between two continuous variables were analyzed using spearman's rank correlation coefficients. The association of the variables with disease specific survival (DSS) and progression-free survival (PFS) after the ICIs therapy was evaluated using the Cox proportional hazards regression model. In the univariate analyses, each variable was included in a Cox regression model alone. In the multivariable analyses, each variable was added to a Cox regression model, and the influence of each variable was assessed. Kaplan-Meier analysis was also performed for DSS and PFS analysis, and the log-rank test was employed. Receiver operating characteristics (ROC) analysis was performed to assess the predictive value of hepatic steatosis for predicting favorable DSS.

Results

Patient Characteristics

Seventy-four gastric cancer patients who were treated with Nivolumab or Pembrolizumab in our institute were retrospectively identified. However, 4 patients missed non-contrast enhanced CT, and therefore; a total of 70 patients were eligible for this study. The subjects included 48 men and 22 women, with a median age of 71.0 years (range 23-87 years). Patients' characteristics were summarized in Table 1. Sixty-five cases were treated with Nivolumab, while five were treated with Pembrolizumab.

Associations with sarcopenia and systemic inflammatory markers

In this cohort, 34 patients were categorized as sarcopenia and 36 were categorized as non-sarcopenia. LSR of patients with sarcopenia was significantly lower than that of non-sarcopenia (1.26 ± 0.12 vs.

1.34 ± 0.14, P=0.02). LSR showed significant negative correlations with NLR, PLR, and MLR (R=-0.346, P=0.003; R=-0.250, P=0.03; R=-0.297, P=0.01; respectively, Fig. 2).

Associations of LSR with serum IGF-1 and GH

Patients were grouped into a high LSR group (LSR≥1.27, median) and a low LSR group (LSR<1.27). Patients of the low LSR group showed significantly higher level of serum IGF-1 than those of the high LSR group, while serum GH didn't show a significant association with LSR (P=0.03, P=0.1, respectively; Table 2).

Correlations of hepatic steatosis, sarcopenia, and inflammatory markers with survival

Table 3 demonstrated univariate and multivariate Cox regression analyses for DSS and PFS. In univariate analysis of DSS, age, LSR, CRP, NLR, PLR, and MLR were significantly correlated with DSS (P=0.01, <0.0001, <0.0001, 0.001, 0.01, 0.0007, respectively), and multivariate analysis demonstrated that LSR was the independent prognostic factor for DSS (P=0.01). In univariate analysis of PFS, age, LSR, CRP, and MLR showed a significant correlation with PFS (P=0.003, <0.0001, 0.007, 0.02, respectively). In multivariate analysis, it was demonstrated that LSR was the independent prognostic factor for PFS (P=0.01). In Kaplan-Meier analyses (Fig. 3), patients with higher LSR (> 1.27, median) showed significantly better DSS and PFS (P<0.0001, P=0.0001, log-rank, respectively).

Prediction of favorable survival using LSR

According to the median overall survival of gastric cancer patients treated with Nivolumab in the ATTRACTION-2 study (5.26 months), patients were grouped into a favorable DSS group (DSS > 5.3 months, 47 patients) and an unfavorable DSS group (DSS ≤ 5.3 months, 23 patients). For this analysis, all patients were eligible, because no patients were censored within 5.3 months from the start of ICIs therapy. ROC analysis demonstrated that AUC for prediction of favorable DSS (> 5.3 months) using LSR was 0.80, which suggested that LSR was a good marker for the prediction of favorable DSS in gastric cancer patients treated with ICIs (Fig. 4). If the cut-off point was set at 1.263 (calculated by Youden Index method), sensitivity was 70.2%, specificity was 82.6%, positive predictive value was

89.1%, and accuracy was 74.2% for prediction of patients who survived more than 5.3 months since the start of ICIs therapy.

Discussion

The link between hepatic steatosis and cancer cachexia has been recently reported [5-8]. Jones et al. recently reported demonstrated that tumor progression triggered hepatic metabolic dysfunction as part of the cancer cachexia, particularly by reduced hepatic very-low-density-lipoprotein (VLDL) secretion and hypobetalipoproteinemia, leading to accumulation of fat in the liver [8]. Given the associations of the cachexia markers such as the loss of skeletal muscle and adipose tissue or the elevation of inflammatory markers with the response to ICIs therapy in cancer [19-21], hepatic steatosis might also be associated with the response to ICIs therapy. In addition, it was reported that ICIs were ineffective against NASH-related HCC in NASH-HCC mouse mode [12]. Therefore, we speculated that the liver fat absolutely played an important role in the metabolic performance during cancer cachexia, and might lead to the resistance to ICIs therapy of cancer.

This is the first clinical report showing prognostic importance of hepatic steatosis in ICIs therapy for advanced gastric cancer patients. Interestingly, our study demonstrated that the severe steatohepatitis was associated with sarcopenia (skeletal muscle loss) and the elevation of inflammatory markers such as NLR, PLR, and LMR, and the worse survival. These results suggested the importance of hepatic steatosis in cancer cachexia, and this cancer cachexia-associated steatohepatitis (CASH) obviously associated with the response to ICIs therapy. Several meta analyses demonstrated that the elevation of these systemic immune-inflammatory markers were associated with poor prognosis in cancer patients treated with ICIs [22,23]. In gastric cancer, Ogata et al. reported that NLR before the treatment may be an effective prognostic factor in patients with gastric cancer treated with Nivolumab [21]. Considering these associations of the systemic immune-inflammatory markers with survival in cancer patients treated with ICIs, our results on the relationship between hepatic steatosis and survival of gastric cancer patients treated with ICIs were quite reasonable. In addition, we found that LSR (hepatic steatosis quantified by CT) was the only independent prognostic factor for both DSS and PFS, compared to other cancer cachexia markers. In NASH patients, a study reported a negative effect of the presence of excess liver fat on the immune system in NASH patients [11], and another study suggested that ICIs were ineffective against NASH-related HCC [12]. The latter study speculated that it was probably because of impaired immune surveillance due to NASH-related exhausted, unconventionally activated T cell [12]. Those studies were consistent with the results of our study, and we believe that hepatic steatosis must have a great impact on ICIs therapy. If these speculations are true, the treatment for NASH may improve the outcome of ICIs therapy for any types of cancers. However, further investigation will be needed to confirm this speculation.

Our study also attempted to predict favorable DSS after ICIs therapy with use of LSR value, and it was found that LSR could be a reasonable marker for the prediction of favorable survival in ICIs therapy of gastric cancer. Considering the high specificity value (82.6%) of LSR for prediction of favorable survival in ICIs therapy, we should need special care for patients whose LSR is less than 1.263, because such patients may fail ICIs therapy in the early period.

However, how can we improve CASH? Our study demonstrated that lower LSR, which suggested more severe hepatic steatosis, was significantly associated with higher level of serum IGF-1. Ichikawa et al. reported that IGF-1 level was significantly higher in the patients who had moderate to severe hepatic steatosis [24], and our result was consistent with their study. It is speculated that higher IGF-1 level induces lower growth hormone (GH) level from negative feedback mechanism between IGF-1 and GH, and, in turn, lower GH level may contribute to severe triglyceride accumulation in hepatocyte leading to severe hepatic steatosis [24]. Therefore, the reduction of IGF-1 may have a positive effect on ICIs treatment of gastric cancer, and we believe that exercise and nutrition can be key factors to improve ICIs therapy for gastric cancer. In fact, a previous report suggested that intense walking exercise could reduce serum IGF-1 [25]. Regarding nutrition, it was reported that dietary factors could alter circulating concentration of IGF-1, with substantial evidence that a high intake of dairy protein is associated with raised circulating IGF-1 [26]. Therefore, exercise and nutritional intervention can be one of the solutions for CASH, and may lead to improvement of ICIs therapy outcome, but we need further investigations to find effective treatments for CASH [27].

Our study has several limitations. First, our findings are based on single-center data, and the sample size was small. Our findings need to be confirmed in multicenter investigations, and a larger patient population should be studied. Second, ROIs for liver and spleen were manually drawn, and this procedure might be relatively subjective. A computerized segmentation method with high reproducibility and reliability should be developed in the future study. Third,

Conclusion

Though this was a relatively small study, it was demonstrated that hepatic steatosis evaluated by CT can be a prognostic biomarker for immunotherapy of gastric cancer, reflecting sarcopenia and systemic inflammation. We think that LSR can be a biomarker with wider availability, because it is measurable even when a patient has no measurable tumor lesion. We believe that our results will provide an important insight into selecting the optimal therapeutic strategy for patients with advanced gastric cancers.

Statement of Ethics

This retrospective study protocol was approved, and the need for additional informed consent to participate in this retrospective study was waived by the Clinical Research Ethics Committee at Chiba University Hospital (IRB number, 3032). All procedures performed in this study were in accordance with the Helsinki declaration.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

Koichi Hayano: study concept and design, ac- quisition of data, statistical analysis, and drafting of the manu- script. Masayuki Kano, Hiroshi Suito Yasunori Matsumoto, Yoshihiro Kurata, Ryota Otsuka, Tetsuro Isozaki, Takeshi Toyozumi, Kentaro Murakami, and Masaya Uesato: acquisition of data. Gaku Ohira and Hisahiro Matsubara: acquisition of data and critical revision.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Figure Legends

Fig. 1. To measure the severity of hepatic steatosis, the mean CT attenuation value of the liver and that of the spleen were obtained using a single oval region-of-interest of about 200 mm2, taking care to avoid vessels, calcifications, and masses on non-contrast enhanced CT.

Fig. 2. LSR showed significant negative correlations with NLR (a), PLR (b), and MLR (c).

Fig. 3. Kaplan-Meier analyses demonstrated that patients with higher LSR (>1.27, median) showed significantly better DSS (a) and PFS (b).

Fig. 4. ROC analysis demonstrated that AUC for prediction of favorable DSS (> 5.3 months) using LSR was 0.80, which suggested that LSR was a good marker for the prediction of favorable DSS in ICIs therapy.

Table 1; Patient characteristics

Patient Demographics	Variables	Value
Sex	Male / Female	48 / 22
Age	Median / range	71.0 / 23-87
Eastern cooperative oncology group performance status	0 / 1	30 / 40
Type of cancer	Recurrence / Unresectable	39 / 31
Number of prior regimens	1 / 2/ 3 / 4	5 / 61 / 2 / 2

Table 2; Association of LSR with serum IGF-1 and GH

	High LSR (≥1.27, median)	Low LSR (< 1.27)	P
IGF-1 (ng/ml)	46.00 ± 24.30	62.97 ± 32.37	0.03*
GH (ng/ml)	4.89 ± 4.90	3.46 ± 3.15	0.1

IGF-1, insulin-like growth factor 1; GH, growth hormone; *Significant difference at $P < 0.05$

Table 3; Univariate and multivariate Cox regression analyses for disease specific survival and progression free survival

Disease specific survival			Univariate			Multivariate
Variables	Categories	HR	95%CI	P	HR	95%CI
Sex	Men vs. Women	0.84	0.460 – 1.561	0.5		
Age	< 71 vs. ≥ 71	0.41	0.207 – 0.829	0.01*	0.65	0.325 – 1.308
LSR	≥ 1.27 vs. < 1.27	3.99	2.018 – 7.891	<0.0001*	2.35	1.173 – 4.707
Sarcopenia	No vs. Yes	1.69	0.913 – 3.137	0.09		
CRP	≥ 0.35 vs. < 0.35	0.26	0.148 – 0.460	<0.0001*	0.30	0.143 – 0.645
NLR	≥ 2.42 vs. < 2.42	0.34	0.178 – 0.658	0.001*	0.74	0.317 – 1.731
PLR	≥ 152.5 vs. < 152.5	0.44	0.237 – 0.852	0.01*	0.81	0.347 – 1.913
MLR	≥ 0.288 vs. < 0.288	0.32	0.165 – 0.618	0.0007*	0.69	0.306 – 1.577
Progression free survival			Univariate			Multivariate
Sex	Men vs. Women	0.95	0.543 – 1.667	0.8		
Age	< 71 vs. ≥ 71	0.40	0.223 – 0.745	0.003*	0.53	0.286 – 1.008
LSR	≥ 1.27 vs. < 1.27	3.08	1.763 - 5.387	<0.0001*	2.42	1.194 – 4.927
Sarcopenia	No vs. Yes	1.08	0.644 – 1.828	0.7		
CRP	≥ 0.35 vs. < 0.35	0.48	0.281 – 0.825	0.007*	0.45	0.222 – 0.941
NLR	≥ 2.42 vs. < 2.42	0.60	0.355 – 1.037	0.06		
PLR	≥ 152.5 vs. < 152.5	0.72	0.420 – 1.234	0.2		
MLR	≥ 0.288 vs. < 0.288	0.54	0.319 – 0.939	0.02*	0.97	0.465 – 2.049

Grouping was based on the median value; LSR, Liver to spleen CT attenuation ratio; CRP, C-reactive protein; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet-to-lymphocyte ratio; MLR, Monocyte-to-lymphocyte ratio; *Significant difference at $P < 0.05$

Figure 1

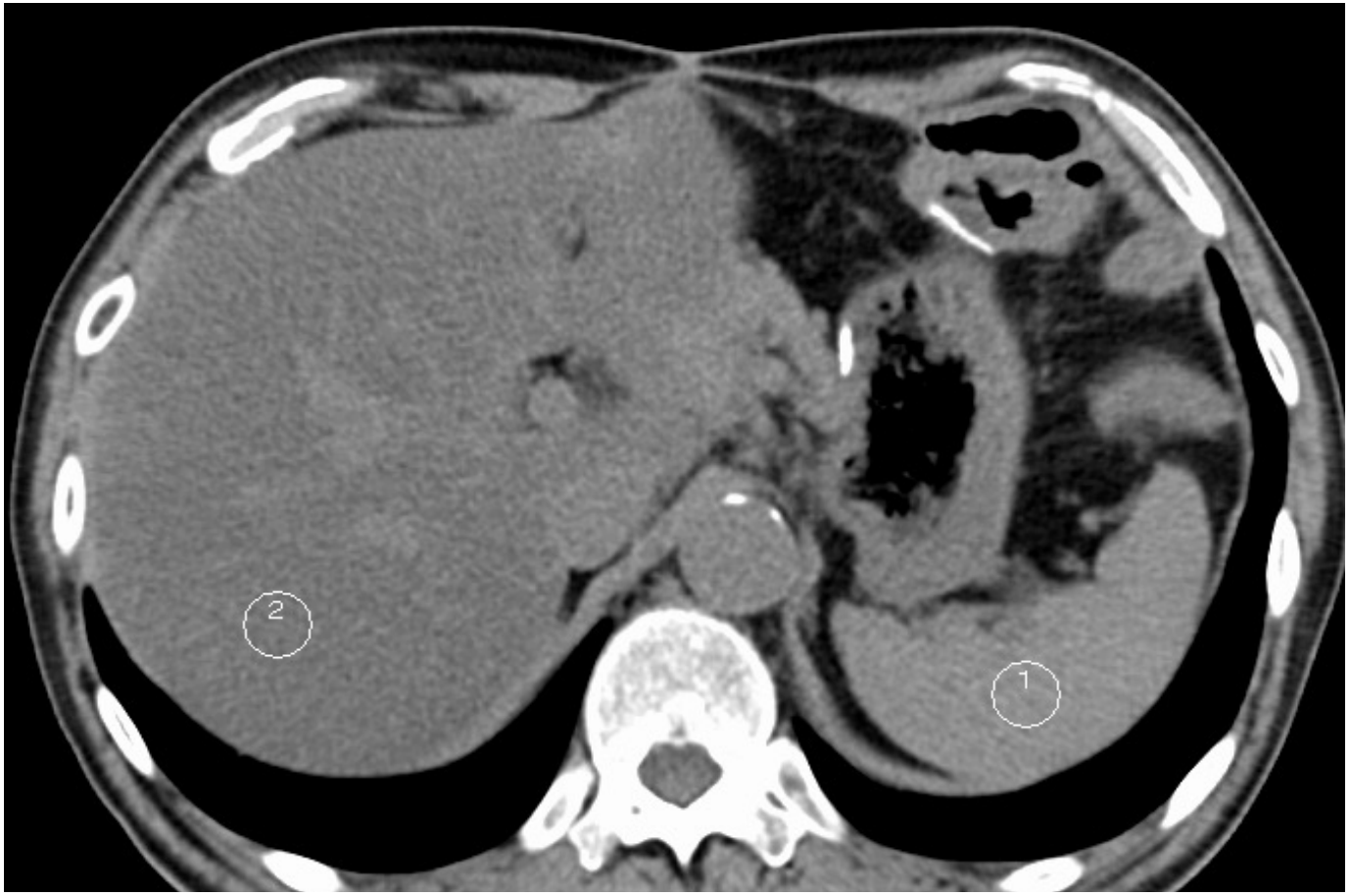
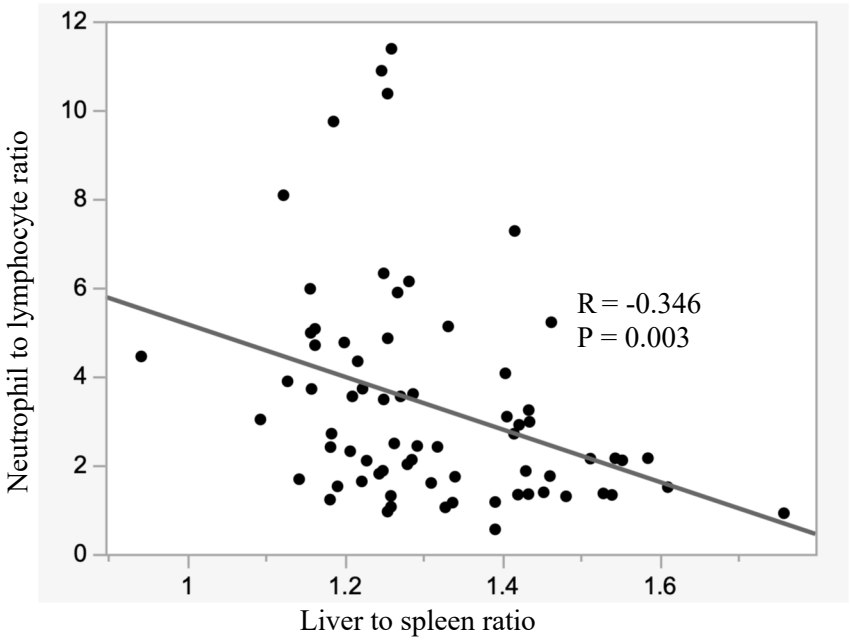
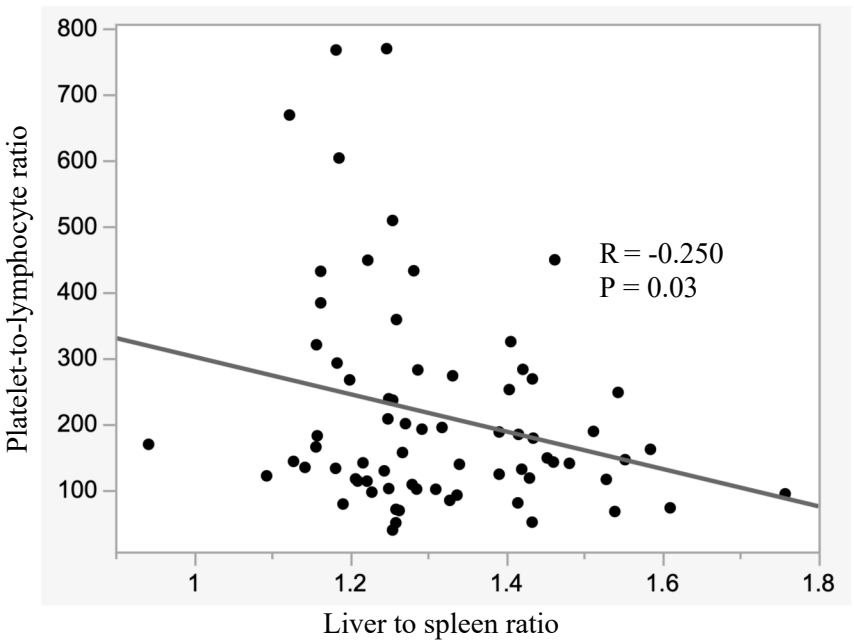


Figure 2

a


Neutrophil to lymphocyte ratio
Liver to spleen ratio
R = -0.346
P = 0.003

b


Platelet-to-lymphocyte ratio
Liver to spleen ratio
R = -0.250
P = 0.03

c

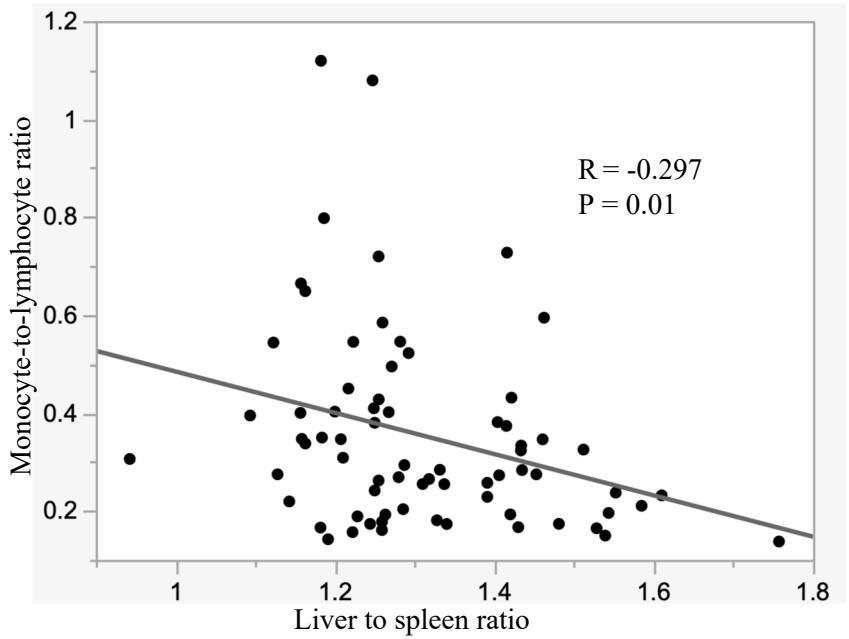
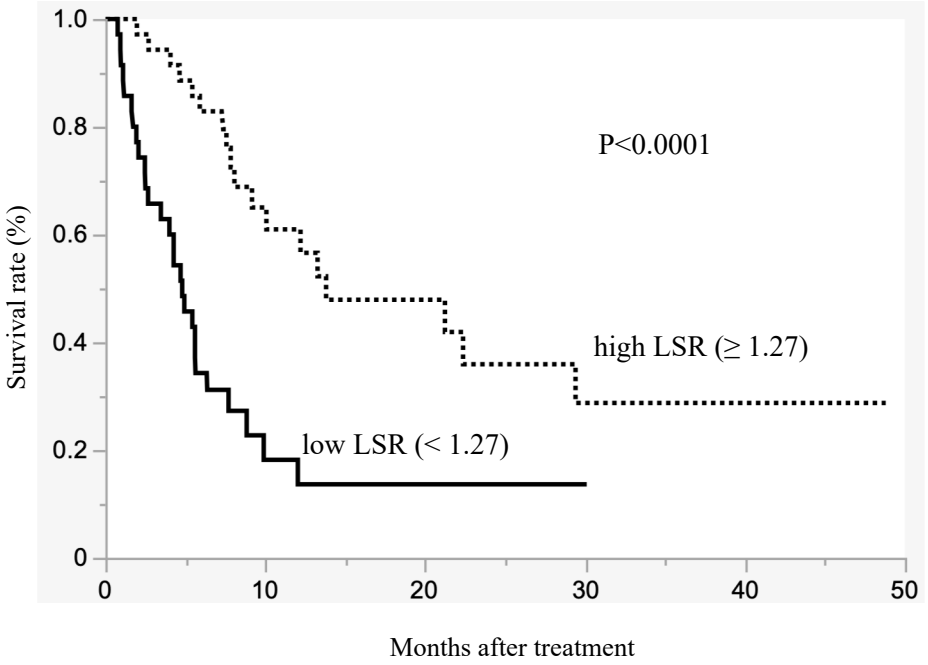

Monocyte-to-lymphocyte ratio
Liver to spleen ratio
R = -0.297
P = 0.01

Figure 3

a


1.0
0.8
0.6
0.4
0.2
0
Survival rate (%)
P<0.0001
high LSR (≥ 1.27)
low LSR (< 1.27)
0
10
20
30
40
50
Months after treatment

b

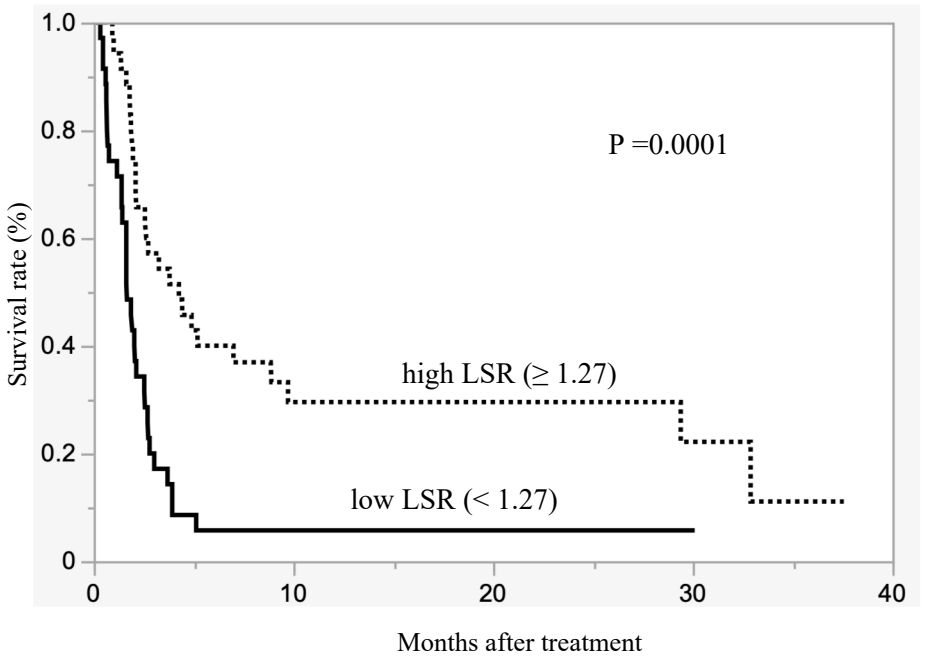
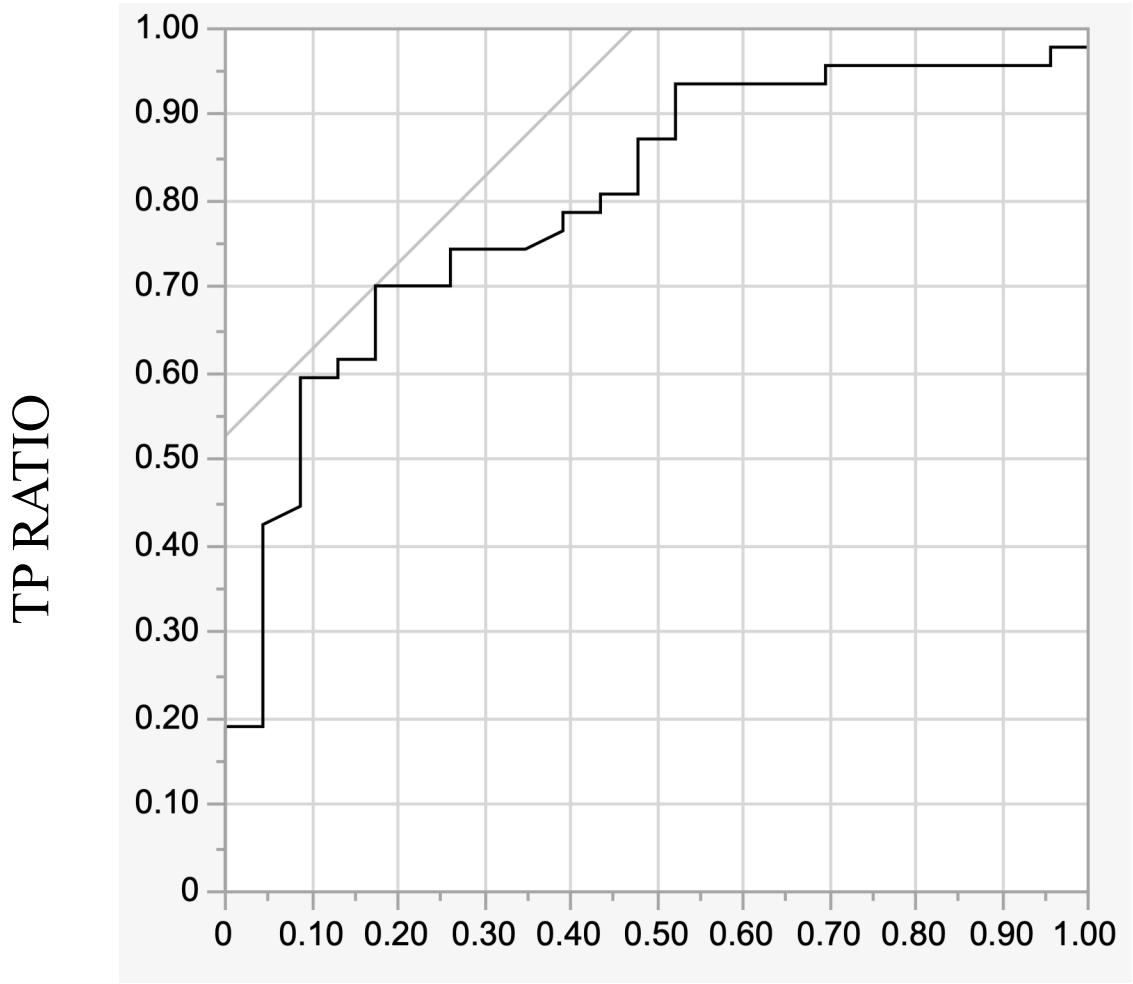

1.0
0.8
0.6
0.4
0.2
0
Survival rate (%)
P =0.0001
high LSR (≥ 1.27)
low LSR (< 1.27)
0
10
20
30
40
Months after treatment

Figure 4


TP RATIO
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
False Positive Ratio