ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN CANCER HORIZONS

ORIGINAL RESEARCH

# Immunological inflammatory biomarkers as prognostic predictors for advanced hepatocellular carcinoma

**M. Nakano*, R. Kuromatsu, T. Niizeki, S. Okamura, H. Iwamoto, S. Shimose, T. Shirono, Y. Noda, N. Kamachi, H. Koga & T. Torimura, for the Kurume Liver Cancer Study Group of Japan**

Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Available online xxx

**Background:** The immunological inflammatory biomarkers for advanced hepatocellular carcinoma are unclear. We aimed to investigate the association of immunity and inflammatory status with treatment outcomes in patients with advanced hepatocellular carcinoma who received molecular-targeted agents as primary treatment.

**Patients and methods:** We enrolled 728 consecutive patients with advanced hepatocellular carcinoma who received sorafenib ($n = 554$) or lenvatinib ($n = 174$) as primary treatment in Japan between May 2009 and June 2020. Changes in the neutrophil-to-lymphocyte ratio before and 1 month after treatment and their impact on survival were evaluated. The cut-off values of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio for predicting overall and progression-free survival were calculated using receiver operating characteristic curves.

**Results:** The neutrophil-to-lymphocyte ratio, but not the platelet-to-lymphocyte ratio, was an independent prognostic factor. Patients with decreased neutrophil-to-lymphocyte ratio survived significantly longer than patients with increased neutrophil-to-lymphocyte ratio (median overall survival: 14.7 versus 10.4 months, $P = 0.0110$). Among patients with a low pre-treatment neutrophil-to-lymphocyte ratio, the overall survival did not differ significantly between those with decreased and those with increased neutrophil-to-lymphocyte ratio after 1 month (median: 19.0 versus 14.8 months, $P = 0.1498$). However, among patients with high pre-treatment neutrophil-to-lymphocyte ratio, those whose neutrophil-to-lymphocyte ratio decreased after 1 month showed significantly longer survival than those whose neutrophil-to-lymphocyte ratio increased (median: 12.7 versus 5.5 months, $P < 0.0001$). The therapeutic effect was not correlated with pre-treatment neutrophil-to-lymphocyte ratio or platelet-to-lymphocyte ratio.

**Conclusions:** The neutrophil-to-lymphocyte ratio is a prognostic factor, along with liver function and tumor markers, in patients with advanced hepatocellular carcinoma who received molecular-targeted agents as primary treatment. Thus, the neutrophil-to-lymphocyte ratio could be a prognostic biomarker for advanced hepatocellular carcinoma primarily treated with immunotherapy.

**Key words:** advanced hepatocellular carcinoma, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, prognosis

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most commonly diagnosed cancer and the third leading cause of cancer-related death worldwide, with an average of 841 080 incident cases and 781 631 deaths reported annually.[1] While early-stage HCC may be curable radically via hepatic resection, radiofrequency ablation, or liver transplantation, there is no curative treatment modality for advanced HCC, explaining its poor prognosis.[2,3]

Molecular-targeted agents (MTAs), such as sorafenib and lenvatinib, are approved as the primary treatment of advanced HCC based on three studies that reported that sorafenib yields superior survival outcomes over placebo [i.e. Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) study[4] and Asia-Pacific study[5]] and that the survival outcomes of lenvatinib are non-inferior to those of sorafenib.[6] However, immunotherapy involving the combination of atezolizumab and bevacizumab resulted in better outcomes over sorafenib as the primary treatment of advanced HCC.[7]

The causal relationship between immunity and inflammatory status with cancer is more widely accepted at present.[8] Moreover, there is strong evidence that cancer-

*Correspondence to: Dr Masahito Nakano, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan. Tel: +81-942-35-3311; Fax: +81-942-34-2623

E-mail: nakano_masahito@kurume-u.ac.jp (M. Nakano).

associated inflammation is a key factor affecting outcomes in these patients.[9] Therefore, the concept of immunity and inflammatory status is considered a critical component of tumor progression.[8] The neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte ratios (PLR) are known simple indicators of immunity and inflammatory status.[10-12] Pretreatment NLR and PLR are also associated with the prognosis of several cancers.[13-16]

Liver function and tumor markers are established prognostic factors in advanced HCC.[17-21] However, the immunological inflammatory biomarkers for advanced HCC are still unclear. Therefore, this study aimed to assess the relationship between real-world treatment outcomes and immunological inflammatory biomarkers among patients with advanced HCC primarily treated with MTAs.

## PATEINTS AND METHODS

### *Study design and patients*

This study was approved by the Ethics Committee of Kurume University (No. 10009, 18146) and the University Hospital Medical Information Network (UMIN) Center (No. UMIN000007427) and was conducted according to the guidelines of the 1975 Declaration of Helsinki. Patients were given comprehensive information regarding the details of the clinical study, and each provided written informed consent before participation.

Since the approval of sorafenib in May 2009 in Japan, 866 patients with advanced HCC have been treated with MTA (sorafenib, $n = 575$; regorafenib, $n = 34$; lenvatinib, $n = 232$; ramucirumab, $n = 25$) in the 19 participating institutions of the Kurume Liver Cancer Study Group of Japan between May 2009 and June 2020. Before the approval of lenvatinib in March 2018 in Japan, 490 patients with advanced HCC were treated with sorafenib as the primary treatment option between May 2009 and February 2018. After its approval, 238 patients with advanced HCC were treated with MTA (sorafenib, $n = 64$; lenvatinib, $n = 174$) as the primary treatment option between March 2018 and June 2020. Here, we prospectively enrolled 728 consecutive patients who were diagnosed with advanced HCC and received sorafenib or lenvatinib as the primary treatment option, with similar eligibility criteria to those in the SHARP[4] and REFLECT studies.[6] Briefly, all enrolled patients met the following requirements: (i) Eastern Cooperative Oncology Group performance status of 0-1[22]; (ii) measurable disease using the Response Evaluation Criteria in Solid Tumors (RECIST)[23]; (iii) Child—Pugh class A or B; (iv) leukocyte count of $\geq 2000/mm^3$; (v) platelet count of $\geq 50 \times 10^9/l$; (vi) hemoglobin level of $\geq 8.5$ g/dl; and (vii) serum creatinine level of $< 1.5$ mg/dl.

### *Diagnosis*

HCC was either confirmed histologically or diagnosed using the noninvasive criteria stipulated by the European Association for the Study of the Liver.[24] Intrahepatic lesions and vascular invasion were diagnosed using a combination of contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (US). Additionally, alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) serum levels were measured up to 1 month before treatment. Intra-abdominal metastases were concurrently assessed during an abdominal CT, MRI, and US for the evaluation of intrahepatic lesions. Pulmonary lesions were detected using chest radiography or CT, which was carried out up to 1 month before treatment. Additional examinations, such as positron emission tomography and brain CT or MRI, were indicated when symptoms attributable to extrahepatic metastasis developed. These examinations were also conducted in cases in which the AFP or DCP levels increased in a manner that could not be explained by the status of the intrahepatic lesions. Liver function was evaluated using both the Child—Pugh class score and albumin-bilirubin (ALBI) grade.[25] Tumor staging was according to the Barcelona Clinic Liver Cancer (BCLC) classification.[26,27]

### *MTA treatment protocol*

The patient's performance status was used to determine the initial MTA dose at the discretion of the chief physician. Discontinuation and dose reductions were allowed based on tolerance. Adverse events were evaluated according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.[28-30] Treatment was discontinued when CTCAE grade $\geq 3$ adverse events occurred.

### *Assessment of tumor response*

Tumor response was evaluated using imaging studies carried out 1 month following the initiation of MTA and every 6-12 weeks thereafter. Tumor markers were evaluated every 4 weeks. The assessment was conducted according to the modified RECIST.[31] Patients who died before their first radiographic assessment were classified as having progressive disease. The time to radiologic progression was defined as the time from MTA initiation to disease progression. Data from patients who died without tumor progression were censored.

### *Statistical analysis*

The primary outcome measure of this study was overall survival (OS), defined as the time from initiation of sorafenib or lenvatinib treatment to the date of death or final follow-up. We used receiver operating characteristic (ROC) curves to calculate the optimal cut-off values for NLR and PLR. Patient characteristics were analyzed using descriptive statistical methods. All variables were calculated using the chi-square test. Univariate and multivariate Cox proportional hazards analyses were carried out to evaluate the interaction between patient characteristics and radiologic progression-free survival (PFS) or OS. Survival curves were generated using the Kaplan—Meier method and compared using the log-rank test and Bonferroni methods. The correlation between therapeutic effects and NLR or PLR was calculated using the *t*-test. The results were expressed as

**Table 1. Baseline patient characteristics ($n = 728$)**

| Characteristics | Value |
|---|---|
| Age (years) | 71.3 ± 9.8<br>72.1 (33.4-94.3) |
| Sex (male/female) | 582 (80%)/146 (20%) |
| Etiology (HBV/HCV/HBV+HCV/both negative) | 126 (17%)/409 (56%)/7 (1%)/186 (26%) |
| Child–Pugh class (A/B)<br>Score (5/6/7/8/9) | 592 (81%)/136 (19%)<br>374 (51%)/218 (30%)/88 (12%)/39 (6%)/9 (1%) |
| ALBI grade (1/2/3) | 210 (29%)/495 (68%)/23 (3%) |
| BCLC stage (B/C) | 277 (38%)/451 (62%) |
| Macrovascular invasion (yes/no) | 165 (23%)/563 (77%) |
| Extrahepatic metastasis (yes/no) | 364 (50%)/364 (50%) |
| AFP (ng/ml) | 11 936 ± 70 352<br>88 (1-987 600) |
| DCP (mAU/ml) | 14 057 ± 75 351<br>487 (2-1 590 000) |
| NLR | 3.71 ± 4.82<br>2.66 (0.31-98.0) |
| PLR | 146.5 ± 105.3<br>117.3 (29.0-1052) |

Results are expressed as the mean ± standard deviation and the median (range) or number (%).
AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; MTA, molecular-targeted agents; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

the mean ± standard deviation (SD), median (range), or number (%). All statistical analyses were carried out using JMP software version 14 (SAS Institute, Inc., Cary, NC). A $P$ value of $<0.05$ was considered statistically significant.

## RESULTS

### Patient characteristics

Of the 728 consecutive patients with advanced HCC, 554 and 174 received sorafenib and lenvatinib, respectively, as the primary MTA treatment. Table 1 shows the patient characteristics. There were 582 (80%) male and 146 (20%) female patients. The mean ± SD and median (range) age were 71.3 ± 9.8 and 72.1 (33.4-94.3) years, respectively. The mean ± SD and median (range) values of pre-treatment NLR were 3.71 ± 4.82 and 2.66 (0.31-98.0), respectively, whereas those of pre-treatment PLR were 146.5 ± 105.3 and 117.3 (29.0-1052), respectively.

### Survival outcomes in the overall cohort

Supplementary Figure S1A, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of Kaplan–Meier analysis of PFS in the overall cohort. The median survival time (MST) was 4.0 months, and the 1-year survival rate was 16%. Supplementary Figure S1B, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of Kaplan–Meier analysis of OS in the overall cohort. The MST was 12.9 months, and the 1-year survival rate was 53%.

### Correlation between OS and pre-treatment NLR or PLR

Pearson's correlation analysis showed that OS had a significant negative correlation with both NLR (correlation coefficient; $R = -0.1377$, $P = 0.0004$; Supplementary Figure S2A, available at https://doi.org/10.1016/j.esmoop.2020.100020) and PLR ($R = -0.1387$, $P = 0.0003$; Supplementary Figure S2B, available at https://doi.org/10.1016/j.esmoop.2020.100020).

### Cut-off values of pre-treatment AFP, DCP, NLR, and PLR for predicting PFS and OS

Supplementary Figure S3A, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of AFP for predicting PFS. The optimal cut-off value was 44.5 ng/ml, with an area under the curve (AUC) of 0.6464. The sensitivity and specificity were 58.9% and 66.0%, respectively. Supplementary Figure S3B, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of AFP for predicting OS. The optimal cut-off value was 360 ng/ml, with an AUC of 0.6483. The sensitivity and specificity were 42.8% and 79.9%, respectively.

Supplementary Figure S3C, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of DCP for predicting PFS. The optimal cut-off value was 130 mAU/ml, with an AUC of 0.5993. The sensitivity and specificity were 67.6% and 52.9%, respectively. Supplementary Figure S3D, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of DCP for predicting OS. The optimal cut-off value was 423 mAU/ml, with an AUC of 0.6313. The sensitivity and specificity were 57.9% and 65.5%, respectively.

Supplementary Figure S3E, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of NLR for predicting PFS. The optimal cut-off value was 2.16, with an AUC of 0.5735. The sensitivity and specificity were 65.0% and 54.0%, respectively. Supplementary Figure S3F, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of NLR for predicting OS. The optimal cut-off value was 3.68, with an AUC of 0.5946. The sensitivity and specificity were 37.3% and 82.0%, respectively.

Supplementary Figure S3G, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off value of PLR for predicting PFS. The optimal cut-off value was 66.8, with an AUC of 0.5643. The sensitivity and specificity were 86.7% and 26.0%, respectively. Supplementary Figure S3H, available at https://doi.org/10.1016/j.esmoop.2020.100020, shows the result of ROC curve analysis for the optimal cut-off values for PLR and OS. The optimal cut-off value was 122.8, with an AUC of 0.5979. The sensitivity and specificity were 50.1% and 65.8%, respectively.

### Prognostic factors for PFS and OS in the overall cohort

Univariate analyses of PFS in the overall cohort identified eight pre-treatment variables as prognostic factors: Child–Pugh class, ALBI grade, BCLC stage, macrovascular invasion,

**Table 2. Univariate and multivariate analyses of the predictive factors for PFS**

| Pre-treatment variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Age (≥72 years) | 1.020 (0.873-1.191) | 0.7998 | | |
| Sex (Male) | 0.881 (0.726-1.069) | 0.2005 | | |
| Etiology (HCV) | 0.898 (0.768-1.051) | 0.1800 | | |
| Child−Pugh class (B) | 1.397 (1.147-1.701) | 0.0009 | 1.198 (0.963-1.490) | 0.1041 |
| ALBI grade (2 or 3) | 1.516 (1.276-1.807) | <0.0001 | 1.290 (1.067-1.561) | 0.0085 |
| BCLC stage (C) | 1.193 (1.015-1.402) | 0.0317 | 1.098 (0.911-1.325) | 0.3238 |
| Macrovascular invasion (yes) | 1.359 (1.129-1.625) | 0.0013 | 1.125 (0.911-1.390) | 0.2712 |
| Extrahepatic metastasis (yes) | 1.120 (0.959-1.309) | 0.1500 | | |
| AFP (≥44.5 ng/ml) | 1.444 (1.231-1.696) | <0.0001 | 1.305 (1.098-1.552) | 0.0025 |
| DCP (≥130 mAU/ml) | 1.495 (1.265-1.771) | <0.0001 | 1.295 (1.081-1.552) | 0.0050 |
| NLR (≥2.16) | 1.407 (1.192-1.666) | <0.0001 | 1.297 (1.073-1.568) | 0.0071 |
| PLR (≥66.8) | 1.408 (1.121-1.792) | 0.0029 | 1.208 (0.927-1.575) | 0.1616 |

AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-gamma-carboxy prothrombin; HCV, hepatitis C virus; HR, hazard ratio; NLR, neutrophil-to-lymphocyte ratio; PFS, progression-free survival; PLR, platelet-to-lymphocyte ratio.

AFP, DCP, NLR, and PLR. In multivariate analyses, ALBI grade 2 or 3, AFP ≥44.5 ng/ml, DCP ≥130 mAU/ml, and NLR ≥2.16 were independent prognostic factors for PFS. All patients with these characteristics had a significantly shorter PFS (Table 2).

Univariate analyses of OS in the overall cohort identified nine pre-treatment variables as prognostic factors: sex, Child−Pugh class, ALBI grade, BCLC stage, macrovascular invasion, AFP, DCP, NLR, and PLR. In multivariate analyses, Child−Pugh class B, ALBI grade 2 or 3, AFP ≥360 ng/ml, DCP ≥423 mAU/ml, and NLR ≥3.68 were independent prognostic factors for OS. All patients with these characteristics had a significantly shorter OS (Table 3).

### *Survival impact of pre-treatment NLR*

Figure 1A shows the results of Kaplan−Meier PFS curves with regard to NLR (cut-off value: 2.16). Patients with NLR <2.16 showed significantly longer MST than those with NLR ≥2.16 (5.3 months versus 3.3 months, $P < 0.0001$). Figure 1B shows the results of Kaplan−Meier OS curves with regard to NLR (cut-off value: 3.68). Patients with NLR <3.68 showed significantly longer MST than those with NLR ≥3.68 (16.0 months versus 7.0 months, $P < 0.0001$).

### *Survival impact of changes in pre-treatment and post-1-month treatment NLR*

Table 4 shows the change in NLR before and 1 month after MTA treatment. The NLR was decreased at 1 month after treatment in 364 (50%) patients, whereas it was increased in 364 (50%) patients. Patients whose pre-treatment NLR was low (median: <2.66) and further decreased after 1 month with MTA were categorized into cohort A ($n = 146$). Patients whose pre-treatment NLR was low (median: <2.66) but increased after 1 month with MTA were categorized into cohort B ($n = 218$). Patients whose pre-treatment NLR was high (median: ≥2.66) but decreased after 1 month with MTA were categorized into cohort C ($n = 218$). Patients whose pre-treatment NLR was high (median: ≥2.66) and further increased after 1 month with MTA were categorized into cohort D ($n = 146$).

Figure 2A shows the results of Kaplan−Meier OS curves with regard to changes in NLR before and 1 month after MTA treatment (decrease or increase). Patients whose NLR was decreased after 1 month showed significantly longer MST than patients whose NLR was increased (14.7 versus 10.4 months, respectively, $P = 0.0110$). Figure 2B shows the results of Kaplan−Meier OS curves in all four cohorts regarding changes in NLR before and 1 month after MTA treatment. The Kaplan−Meier curves showed significant differences in OS in all four cohorts according to the changes in NLR before and 1 month after MTA treatment (all $P < 0.0001$, Figure 2B). Among patients with lower pre-treatment NLR (median: <2.66; cohorts A and B), there was no significant difference in OS between cohort A and cohort B (19.0 versus 14.8 months, $P = 0.1498$). Among those with higher pre-treatment NLR (median: ≥2.66; cohorts C and D), OS was significantly longer in cohort C than in cohort D (12.7 versus 5.5 months, $P < 0.0001$).

### *Association between therapeutic effect and pre-treatment NLR or PLR*

The therapeutic effect (each therapeutic effect, response, and disease control) was not correlated with pre-treatment NLR (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2020.100020) or with pre-treatment PLR (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2020.100020).

## DISCUSSION

Peripheral blood parameters, including white blood cell, neutrophil, lymphocyte, and platelet counts, and indicators of immunity and inflammatory status (i.e. NLR and PLR) have been widely proposed as prognostic factors for many malignancies.[32] NLR and PLR can only be calculated by complete blood count, which involves a few omissions; therefore, it is suitable for a multicenter study. In HCC, several studies have shown that the indicators of immunity and inflammatory status are associated with prognosis.[16,33-36]

In this study, we first investigated the real-world treatment outcomes of patients with advanced HCC who

**Table 3. Univariate and multivariate analyses of the predictive factors for OS**

| Pre-treatment variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR (95% CI) | *P* value | HR (95% CI) | *P* value |
| Age (≥72 years) | 1.138 (0.960-1.351) | 0.1350 | | |
| Sex (male) | 0.783 (0.635-0.965) | 0.0218 | 0.853 (0.682-1.068) | 0.1673 |
| Etiology (HCV) | 0.975 (0.822-1.159) | 0.7796 | | |
| Child—Pugh class (B) | 2.017 (1.639-2.481) | <0.0001 | 1.551 (1.234-1.950) | 0.0002 |
| ALBI grade (2 or 3) | 1.809 (1.487-2.215) | <0.0001 | 1.337 (1.078-1.668) | 0.0089 |
| BCLC stage (C) | 1.281 (1.067-1.538) | 0.0078 | 0.983 (0.795-1.215) | 0.8741 |
| Macrovascular invasion (yes) | 1.472 (1.208-1.784) | 0.0002 | 1.150 (0.913-1.444) | 0.2303 |
| Extrahepatic metastasis (yes) | 1.147 (0.967-1.363) | 0.1149 | | |
| AFP (≥360 ng/ml) | 2.079 (1.744-2.474) | <0.0001 | 1.941 (1.602-2.349) | <0.0001 |
| DCP (≥423 mAU/ml) | 1.857 (1.561-2.212) | <0.0001 | 1.465 (1.214-1.769) | <0.0001 |
| NLR (≥3.68) | 1.950 (1.624-2.335) | <0.0001 | 1.845 (1.463-2.324) | <0.0001 |
| PLR (≥122.8) | 1.435 (1.205-1.709) | <0.0001 | 0.976 (0.786-1.210) | 0.8307 |

AFP, alpha-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; DCP, des-gamma-carboxy prothrombin; HCV, hepatitis C virus; HR, hazard ratio; OS, overall survival; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

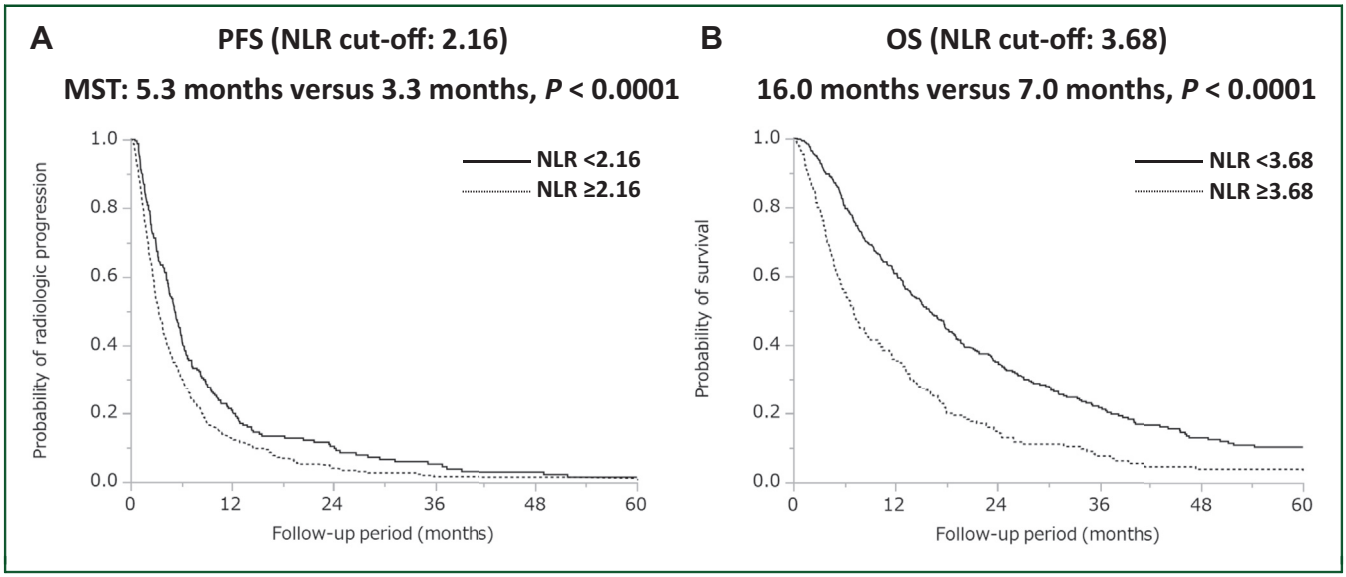


**Figure 1. Survival outcomes according to pre-treatment NLR.**
(A) Kaplan—Meier analysis with log-rank test of PFS according to NLR (cut-off: 2.16). The MST was 5.3 months for those with NLR <2.16 (solid line), whereas it was 3.3 months for those with NLR ≥2.16 (dotted line) ($P < 0.0001$). (B) Kaplan—Meier analysis with log-rank test of OS according to NLR (cut-off: 3.68). The MST was 16.0 months for those with NLR <3.68 (solid line), whereas it was 7.0 months for those with NLR ≥3.68 (dotted line) ($P < 0.0001$).
MST, median survival time; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival.

received MTA as primary treatment. The median PFS and OS were 4.0 and 12.9 months, respectively. In the REFLECT study, the PFS and OS were 3.7 and 12.3 months, respectively, in the sorafenib cohort and 7.4 and 13.6 months, respectively, in the lenvatinib cohort.[6] Considering the proportion of patients who received sorafenib or lenvatinib for advanced HCC, the findings of this study validate those of the REFLECT study that sorafenib or lenvatinib should be the primary treatment of advanced HCC.

**Table 4. Patient grouping according to change in NLR before and 1 month after treatment with MTA**

| Pre-treatment NLR | Decreased ($n = 364$) | Increased ($n = 364$) |
|---|---|---|
| Low ($n = 364$) | 146 (20%) = cohort A | 218 (30%) = cohort B |
| High ($n = 364$) | 218 (30%) = cohort C | 146 (20%) = cohort D |

MTA, molecular-targeted agents; NLR, neutrophil-to-lymphocyte ratio.

Second, we assessed the prognostic factors for patients with advanced HCC treated with MTA and identified liver function, tumor markers, and NLR, but not PLR, as independent prognostic factors for OS and PFS. This could possibly be attributed to the influence of the platelet count. In general, most patients with advanced HCC also have liver cirrhosis. Here, approximately 50% of the enrolled patients had liver cirrhosis, as indicated by a Child—Pugh score of ≥6. These patients had decreased platelet count, and the decreased PLR in the large number of patients may have caused the finding that it is not an independent prognostic factor for both PFS and OS.

Third, we assessed the effects of immunity and inflammatory status on survival outcomes. The optimal NLR cut-off for predicting PFS was 2.16, and patients with an NLR of <2.16 showed significantly better PFS than those with an NLR of ≥2.16. Similar results were obtained for OS. At an optimal NLR cut-off of 3.68, patients with lower NLR

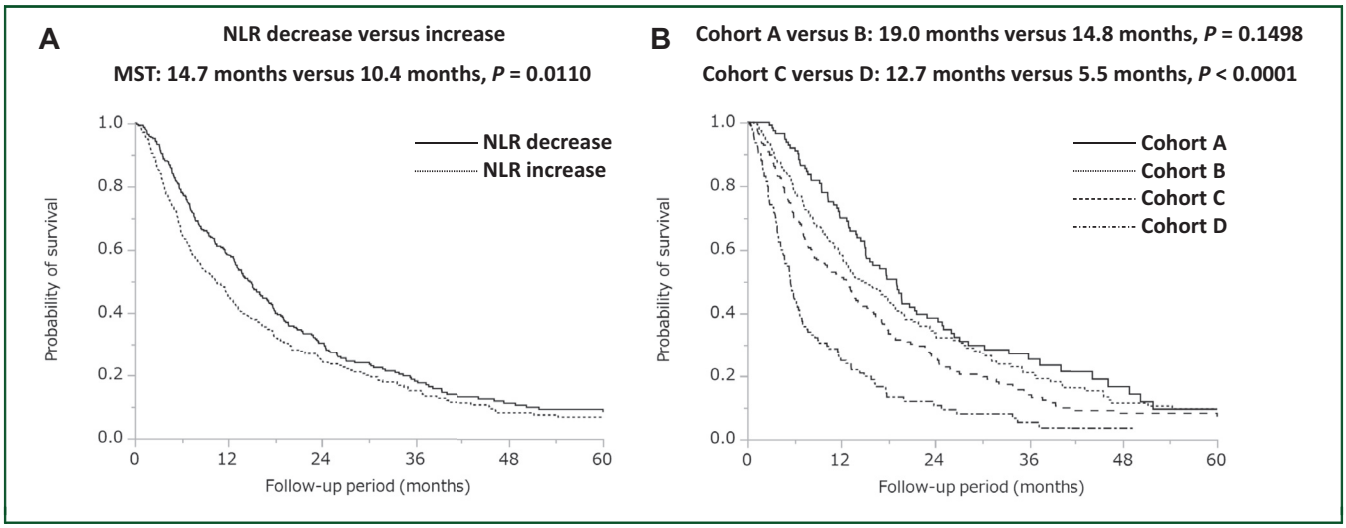


**Figure 2. Survival outcomes according to changes in NLR before and after 1-month treatment with MTA.**
(A) Kaplan—Meier analysis with log-rank test of OS according to change in NLR before and 1 month after treatment with MTA. The MST was 14.7 months for those whose NLR was decreased (solid line), whereas it was 10.4 months for those in whom it was increased (dotted line) ($P = 0.0110$). (B) Kaplan—Meier analysis with log-rank test and Bonferroni test of OS according to change in NLR before and 1 month after treatment with MTA (all $P < 0.0001$). The MST was higher in cohort A (solid line) than in cohort B (dotted line), but the difference was not significant (19.0 versus 14.8 months, $P = 0.1498$). Meanwhile, the MST was significantly higher in cohort C (dashed line) than in cohort D (dash and dotted line) (12.7 versus 5.5 months, $P < 0.0001$).
MST, median survival time; MTA, molecular-targeted agents; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival.

exhibited significantly better OS than those with higher NLR. Collectively, these findings indicated that pre-treatment NLR was a predictor of both PFS and OS in patients with advanced HCC treated with MTA. Additionally, patients whose NLR decreased 1 month after MTA treatment exhibited significantly better OS than those whose NLR increased. This shows that changes in NLR from baseline to 1 month after MTA treatment predict OS. Therefore, we divided the patients into four groups according to whether the NLR was low or high at pre-treatment and whether it was decreased or increased 1 month after MTA treatment. There was no significant difference in OS between those with low pre-treatment NLR (cohorts A and B). Conversely, among those with high pre-treatment NLR (cohorts C and D), OS was significantly higher in patients who showed an increase in NLR at 1 month after MTA treatment than in patients who showed a decrease. A high NLR indicates low immunity or high inflammatory state.[36-40] An improved post-treatment NLR from MTA (i.e. a high pre-treatment NLR that decreases after 1 month of treatment) indicates improved immunity or inflammation, which translates to better OS. A low NLR indicates high immunity or low inflammatory state, and thus MTA has minimal OS benefit in these patients.

Fourth, we assessed the impact of immunity and inflammatory status on the therapeutic effect of MTA as primary treatment of advanced HCC. There were no significant differences in therapeutic effects between those with low and high NLR or between those with low and high PLR. Both NLR and PLR could not predict the therapeutic effect. As we reported, there was no significant difference in OS between those who received sorafenib and who received lenvatinib despite the significantly higher therapeutic effects of lenvatinib.[41] These results indicated that the therapeutic effect is unsuitable for predicting OS in patients with advanced HCC who receive MTA for primary treatment.[41] However, aside from liver function and tumor markers, immune and inflammatory markers (i.e. NLR) were also prognostic factors in patients with advanced HCC treated with MTA.

This study has some limitations. First, the primary treatment (sorafenib or lenvatinib) was selected at the discretion of the chief physician and was not randomized after lenvatinib approval in Japan, resulting in a selection bias for patients with advanced HCC treated with MTA. Second, no further investigations were conducted after the secondary treatment. This study mainly focused on pre-treatment biomarkers for advanced HCC. Further studies on the impact of secondary treatment in OS are needed.

In conclusion, NLR, but not PLR, as an indicator of immunity and inflammatory status, is a prognostic factor along with liver function and tumor markers in patients with advanced HCC receiving MTA as the primary treatment. Specifically, changes in NLR before and 1 month after treatment with MTA predict prognosis. Thus, NLR might be a prognostic biomarker in patients with advanced HCC undergoing immunotherapy as primary treatment.

## ACKNOWLEDGEMENTS

The authors thank the staff of the Kurume Liver Cancer Study Group of Japan; Asakura Medical Association Hospital, Chikugo City Hospital, Iwamoto Clinic, Kurume Central Hospital, Kurume General Hospital, Kurume University Hospital, Kurume University Medical Center, Kyushu Medical Center, Nagata Hospital, Ōmuta City Hospital, Saga Central Hospital, Saiseikai Futsukaichi Hospital, Saiseikai Ōmuta Hospital, Social Insurance Tagawa Hospital, St.

Mary's Hospital, Tobata Kyoritsu Hospital, Yame General Hospital, Yanagawa Hospital, and Yokokura Hospital, for their valuable support. We also thank Editage (www.editage.jp) for English language editing.

## FUNDING

None declared.

## DISCLOSURE

The authors have declared no conflicts of interest.

## REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424.
2. Grandhi MS, Kim AK, Ronnekleiv-Kelly SM, Kamel IR, Ghasebeh MA, Pawlik TM. Hepatocellular carcinoma: from diagnosis to treatment. *Surg Oncol*. 2016;25:74-85.
3. Hartke J, Johnson M, Ghabril M. The diagnosis and treatment of hepatocellular carcinoma. *Semin Diagn Pathol*. 2017;34:153-159.
4. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359:378-390.
5. Cheng AL, Kang YK, Chen Z, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2009;10:25-34.
6. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet*. 2018;391:1163-1173.
7. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382:1894-1905.
8. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420:860-867.
9. Guthrie GJ, Charles KA, Roxburgh CS, Horgan PG, McMillan DC, Clarke SJ. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol*. 2013;88:218-230.
10. Gasparyan AY, Ayvazyan L, Mukanova U, Yessirkepov M, Kitas GD. The platelet-to-lymphocyte ratio as an inflammatory marker in rheumatic diseases. *Ann Lab Med*. 2019;39:345-357.
11. Paliogiannis P, Satta R, Deligia G, et al. Associations between the neutrophil-to-lymphocyte and the platelet-to-lymphocyte ratios and the presence and severity of psoriasis: a systematic review and meta-analysis. *Clin Exp Med*. 2019;19:37-45.
12. Mazza MG, Lucchi S, Tringali AGM, Rossetti A, Botti ER, Clerici M. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in mood disorders: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;84:229-236.
13. Guan Y, Xiong H, Feng Y, Liao G, Tong T, Pang J. Revealing the prognostic landscape of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in metastatic castration-resistant prostate cancer patients treated with abiraterone or enzalutamide: a meta-analysis. *Prostate Cancer Prostatic Dis*. 2020;23:220-231.
14. Liu XB, Gao ZY, Zhang QH, et al. Preoperative neutrophil lymphocyte ratio can be used as a predictor of prognosis in patients with adenocarcinoma of the esophagogastric junction: a systematic review and meta analysis. *Front Oncol*. 2020;10:178.
15. Sunakawa Y, Yang D, Cao S, et al. Immune-related genes to dominate neutrophil-lymphocyte ratio (NLR) associated with survival of cetuximab treatment in metastatic colorectal cancer. *Clin Colorectal Cancer*. 2018;17:e741-e749.
16. Aino H, Sumie S, Niizeki T, et al. The systemic inflammatory response as a prognostic factor for advanced hepatocellular carcinoma with extrahepatic metastasis. *Mol Clin Oncol*. 2016;5:83-88.
17. Yau T, Yao TJ, Chan P, et al. The significance of early alpha-fetoprotein level changes in predicting clinical and survival benefits in advanced hepatocellular carcinoma patients receiving sorafenib. *Oncologist*. 2011;16:1270-1279.
18. Kuzuya T, Ishigami M, Ishizu Y, et al. Early clinical response after 2 weeks of sorafenib therapy predicts outcomes and anti-tumor response in patients with advanced hepatocellular carcinoma. *PLoS One*. 2015;10:e0138776.
19. Reig M, Rimola J, Torres F, et al. Postprogression survival of patients with advanced hepatocellular carcinoma: rationale for second-line trial design. *Hepatology*. 2013;58:2023-2031.
20. Lai Q, Iesari S, Levi Sandri GB, et al. Des-gamma-carboxy prothrombin in hepatocellular cancer patients waiting for liver transplant: a systematic review and meta-analysis. *Int J Biol Markers*. 2017;32:e370-e374.
21. Shimizu D, Inokawa Y, Sonohara F, Inaoka K, Nomoto S. Search for useful biomarkers in hepatocellular carcinoma, tumor factors and background liver factors (Review). *Oncol Rep*. 2017;37:2527-2542.
22. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5:649-655.
23. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228-247.
24. Bruix J, Sherman M, Llovet JM, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol*. 2001;35:421-430.
25. Johnson PJ, Berhane S, Kagebayashi C, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol*. 2015;33:550-558.
26. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis*. 1999;19:329-338.
27. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis*. 2010;30:61-74.
28. Liu YJ, Zhu GP, Guan XY. Comparison of the NCI-CTCAE version 4.0 and version 3.0 in assessing chemoradiation-induced oral mucositis for locally advanced nasopharyngeal carcinoma. *Oral Oncol*. 2012;48:554-559.
29. Basch E, Reeve BB, Mitchell SA, et al. Development of the National Cancer Institute's patient-reported outcomes version of the common terminology criteria for adverse events (PRO-CTCAE). *J Natl Cancer Inst*. 2014;106:dju244.
30. Atkinson TM, Ryan SJ, Bennett AV, et al. The association between clinician-based common terminology criteria for adverse events (CTCAE) and patient-reported outcomes (PRO): a systematic review. *Support Care Cancer*. 2016;24:3669-3676.
31. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis*. 2010;30:52-60.
32. Chen L, Kong X, Yan C, Fang Y, Wang J. The research progress on the prognostic value of the common hematological parameters in peripheral venous blood in breast cancer. *Onco Targets Ther*. 2020;13:1397-1412.
33. Ji F, Liang Y, Fu SJ, et al. A novel and accurate predictor of survival for patients with hepatocellular carcinoma after surgical resection: the neutrophil to lymphocyte ratio (NLR) combined with the aspartate aminotransferase/platelet count ratio index (APRI). *BMC Cancer*. 2016;16:137.
34. Casadei Gardini A, Scarpi E, Faloppi L, et al. Immune inflammation indicators and implication for immune modulation strategies in advanced hepatocellular carcinoma patients receiving sorafenib. *Oncotarget*. 2016;7:67142-67149.
35. Liu L, Gong Y, Zhang Q, Cai P, Feng L. Prognostic roles of blood inflammatory γ markers in hepatocellular carcinoma patients taking sorafenib. A systematic review and meta-analysis. *Front Oncol*. 2019;9:1557.

36. Li S, Feng X, Cao G, Wang Q, Wang L. Prognostic significance of inflammatory indices in hepatocellular carcinoma treated with transarterial chemoembolization: a systematic review and meta-analysis. *PLoS One*. 2020;15:e0230879.
37. Wang C, He W, Yuan Y, et al. Comparison of the prognostic value of inflammation-based scores in early recurrent hepatocellular carcinoma after hepatectomy. *Liver Int*. 2020;40:229-239.
38. Ishibashi Y, Tsujimoto H, Yaguchi Y, Kishi Y, Ueno H. Prognostic significance of systemic inflammatory markers in esophageal cancer: systematic review and meta-analysis. *Ann Gastroenterol Surg*. 2020;4:56-63.
39. Itoh S, Yugawa K, Shimokawa M, et al. Prognostic significance of inflammatory biomarkers in hepatocellular carcinoma following hepatic resection. *BJS Open*. 2019;3:500-508.
40. Casadei Gardini A, Foschi FG, Conti F, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. *Dig Liver Dis*. 2019;51:681-688.
41. Nakano M, Kuromatsu R, Niizeki T, et al. Primary treatment with molecular-targeted agents for hepatocellular carcinoma: a propensity score-matching analysis. *Hepatol Commun*. 2020;4:1218-1228.