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Evaluation of prognostic values of inflammation-based makers in patients with HBV-related acute-on-chronic liver failure

Junjun Cai, PhD[a], Kai Wang, PhD[b], Tao Han, MD, PhD[c,*], Huiqing Jiang, MD, PhD[a,*]

Abstract

Systemic inflammatory responses are associated with the development and progression of liver failure. Neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), prognostic nutritional index (PNI), red cell distribution width (RDW), RDW-to platelet ratio (RPR), mean platelet volume (MPV), and MPV-to platelet ratio (MPR) are markers of systemic inflammation. This study aimed to evaluate the prognostic values of these inflammatory markers in patients with hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF).

203 HBV-ACLF patients, 79 cirrhosis patients (LC), 63 chronic hepatitis B (CHB), and 81 healthy subjects (HS) participated in this cohort study. Complete blood counts and biochemical examinations were obtained after overnight fasting. Multivariate analyses of 90-day outcome predictors were analyzed by Cox regression models. Survival probability curves were calculated by the Kaplan–Meier method.

The levels of NLR, MLR, RDW, MPV, RPR, and MPR were significantly higher and PNI was lower in patients with liver failure at presentation compared to those in LC, CHB, and HS ($P<.001$). In acute-on-chronic liver failure (ACLF) patients, NLR and MLR were higher in nonsurvivors than in survivors ($P<.001$), while other inflammatory markers showed no difference. ROC curve analyses showed that NLR combined with MLR had the highest AUC for identified poor outcome, followed by NLR, chronic liver failure-sequential organ failure assessment (CLIF-SOFA), MLR, model for end-stage liver disease (MELD), Child-Turcotte-Pugh (CTP) and TBIL. Multivariate analyses showed that TBIL, NLR, CTP, MELD, and CLIF-SOFA were independent predictors for 90-day mortality.

Combination of NLR and MLR are more accurate prognostic markers for predicting poor outcome than either marker alone in ACLF patients. And this combination is superior to the CLIF-SOFA, MELD, CTP score, and TBIL in terms of prognostic ability.

Abbreviations: ACLF = acute-on-chronic liver failure, CLIF-SOFA = chronic liver failure-sequential organ failure assessment, CTP = Child-Turcotte-Pugh, MELD = model for end-stage liver disease, MLR = monocyte-to-lymphocyte ratio, MPR = MPV-to platelet ratio, MPV = mean platelet volume, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, PNI = prognostic nutritional index, RDW = red cell distribution width, RPR = RDW-to platelet ratio.

Keywords: hepatitis B, liver failure, prognosis, systemic inflammatory response

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1. Introduction

Acute-on-chronic liver failure (ACLF) is a devastating condition characterized by the acute deterioration of liver function over a short period of time under various precipitating events.[1] In China, about 82% cases of ACLF are caused by hepatitis B virus (HBV) infection.[2] The prognosis of ACLF is extremely poor due to resulting multisystem organ failure if liver transplantation is not available. Therefore, finding an objective, accurate, user-friendly, inexpensive and reproducible marker for ACLF prognosis and disease monitoring is urgently needed.

Currently, a series of predictive scoring systems are available for stratifying the severity of the condition and assessing the prognosis in patients with ACLF. These include Child-Turcotte-Pugh (CTP) score, model for end-stage liver disease (MELD) score, MELD-sodium (MELD-Na) score, chronic liver failure-sequential organ failure assessment (CLIF-SOFA) score, and CLIF Consortium ACLF (CLIF-C-ACLF) score.[1,3–6] However, due to the large number of parameters needed to calculate these scores, as well as their low sensitivity, these systems are too complex to provide quick patient evaluations and are not practical for clinicians. For example, the CTP score comes with limitations: subjective criteria such as hepatic encephalopathy and ascites are included in the scoring and the score range of disease severity (7–15) is narrow.[3] Due to the involvement of the logarithms in calculating the MELD score, clinicians have to use

an online calculator.[4] The CLIF-SOFA score includes 6 components (liver, kidney, brain, coagulation, circulation, and lungs) with sub-scores ranging from 0 to 4 to stratify the severity of the disease.[1] In addition, most predictive systems fail to take systemic inflammatory response into consideration, which is closely associated with the progression of liver failure and related cirrhotic complications.[1,3–6] As a result, research is now focusing on finding new simple and reliable markers.

Currently, an accumulation of evidence points to systemic inflammatory responses being correlated with ACLF development and progression.[7,8] Activation of systemic inflammation is characterized by an excessively proinflammatory cytokine profile which is believed to mediate hepatic inflammation, apoptosis, and necrosis of hepatocytes.[9] Hepatocyte necrosis may trigger a complicated immune response that includes the emigration of granulocytes from the bone marrow into the peripheral blood.[10] Additionally, an excessive immune activation may result in a reduction of lymphocytes numbers caused by impaired lymphopoiesis and cell necrosis.[10,11] Moreover, it is reported that inflammation may influence bone marrow function and iron metabolism. Inflammatory cytokines may suppress erythrocyte maturation and induce larger, newer reticulocytes to enter the peripheral blood which may result in increased RDW values.[12] An increasing amount of studies have demonstrated that peripheral blood neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), prognostic nutritional index (PNI), red cell distribution width (RDW), RDW-to platelet ratio (RPR), mean platelet volume (MPV), and MPV-to platelet ratio (MPR) are indicators of systematic inflammatory response and are widely investigated as useful predictors of the clinical outcomes in various diseases.[13–15]

In this study, we aimed to evaluate the prognostic values of these inflammation-based makers in patients with HBV-related ACLF.

2. Materials and methods

2.1. Patient selection

We recruited 203 patients with hepatitis B-related ACLF, 79 patients with hepatitis B-related Child-Pugh A cirrhosis (LC), 63 patients with chronic hepatitis B (CHB) and 81 healthy subjects (HS). ACLF was defined as the acute deterioration of liver function manifested as jaundice [total bilirubin (TBIL) ≥5 mg/dL or ≥85umol/L] and coagulopathy with international normalized ratio of prothrombin time (INR) ≥1.5 or prothrombin activity (PTA) ≤40%], complicated with ascites and/or hepatic encephalopathy noted within 4 weeks in a patient diagnosed with HBV-related cirrhosis.[16] All patients were recruited between March 2011 and February 2014 at the Tianjin Third Central Hospital. Exclusion criteria were as follows: co-infection with human immunodeficiency virus, hepatitis A, C, D, and E viruses or other hepatitis viruses, autoimmune diseases, alcoholic liver disease, drug-induced liver injury, coexistent hepatocellular carcinoma, and any other serious medical illness or patients who had received any immunotherapy. Patients with cardiac diseases, endocrinological disorders, hematological disease and other types of cancer were also excluded.

Once admitted, all patients were given a standard medical treatment including antiviral treatment, intravenous infusion albumin and plasma, appropriate energy support, and the treatment of any complications.

The study was approved by the Institutional Ethics Committee of Tianjin Third Central Hospital and performed in adherence with the principles of the Declaration of Helsinki. Informed consent was obtained from the patients or their family members.

2.2. Laboratory analysis

Blood samples were collected from an antecubital vein after overnight fasting. Complete blood counts including white blood cell count (WBC), platelet (PLT) neutrophil count, lymphocyte count, monocyte count, and other parameters were analyzed using a Beckman-Coulter LH 750 (Brea, CA). Albumin (ALB), total bilirubin (TBIL) and creatinine (Cr) were determined using a Fully Automatic Biochemical Analyzer (AU2700, Olympus, Tokyo, Japan). The international normalized ratio (INR) was assessed using a Fully Automatic Coagulometer (Beckman-Coulter lnc., CA). HBV DNA levels in the serum of patients were quantified by ABI ViiA7TM Sequence Detection System (Life Technologies, Connecticut), with a lower limit of detection of $2.70\log_{10}$ copies/ml (500copies/mL). Demographic and clinical characteristics were collected from the patients' electronic medical records. The MELD score was calculated using the Malinchoc formula: MELD score = $3.78 \times$ loge [bilirubin (mg/dL) + $11.2 \times$ loge (INR) + $9.57 \times$ loge [creatinine (mg/dL)] + 6.43.[4] The CLIF-SOFA score was created by the European Associated for the Study of the Liver-Chronic Liver Failure (EASL-CLIF) Consortium and includes 6 components (liver, kidney, brain, coagulation, circulation, and lung) and each with sub-scores ranging from 0 to 4 to stratifying the severity of the disease.[1] PNI = albumin (g/L) + $5 \times$ lymphocyte count (10^9/L).[15]

2.3. Statistical analysis

Normally distributed variables were expressed as means ± standard deviation (SD), and non-normally distributed variables were expressed as a median and interquartile range (IQR). Count and percentages were used to describe categorical variables. Two independent groups were compared using the t test for continuous normally-distributed variables and the Mann–Whitney U test for non-normally distributed variables. If more than 2 groups were compared, we used the 1-way analysis of variance (ANOVA) and Kruskal–Wallis tests, respectively. For categorical variables, comparisons between groups were made using the Chi-squared tests, or the Fisher test as appropriate. The Kaplan–Meier method was used to calculate the 90-day survival probability curves, which were compared with the log-rank test. Cox regression models were used for multivariate analysis of outcome predictors. Cut-off values for the identification of non-survivors with HBV-related ACLF and survivors were determined using the receiver operating characteristic (ROC) analysis. All calculations were performed using SPSS software, version 17.0 (SPSS Inc., Chicago, IL), GraphPad PRISM 5.02 software (GraphPad Software, San Diego, CA) and MedCalc (MedCalc Software, Ostend, Belgium). All the P values <.05 based on a 2-tailed test were considered statistically significant.

3. Results

3.1. Patient characteristics

The demographic and clinical characteristics of patients were summarized in Table 1. The 90-day mortality of ACLF group was

Table 1

Characteristics of all recruited subjects.

Variable	HS N=81	CHB N=63	LC N=79	ACLF N=203	*P*-value
Age (years)*	39.69±14.41	37.97±13.96	48.27±11.90	51.14±11.77	<.001
Gender (M:F)*	53/28	55/18	58/21	151/52	.058
ALB (g/L)*	44.61±2.36	39.68±3.76	38.17±6.51	29.05±5.33	<.001
TBIL (μmol/L)*	12.22 (9.52–14.11)	17.28 (11.12–24.85)	17.40 (11.80–24.00)	203.50 (122.60–321.30)	<.001
Cr (μmol/L)*	55.00 (44.25–65.60)	67.80 (57.48–77.38)	64.55 (53.98–73.05)	62.00 (51.00–83.00)	<.001
INR*	NA	1.02 (0.98–1.11)	1.16 (1.10–1.26)	2.14 (1.72–2.61)	<.001
HBV-DNA-log$_{10}$ (IU/mL)[†]	0	6.49 (4.70–7.73)	5.44 (4.45–6.66)	5.81 (4.61–7.22)	.152
HBeAg positive-n (%)[†]	0	40 (63.4)	36 (45.6)	117 (57.6)	.076
PLT (10^9/L)*	225.00 (188.00–266.50)	177.00 (133.00–215.00)	71.00 (46.00–115.00)	74.00 (52.00–115.00)	<.001
WBC (10^9/L)*	5.05 (4.51–5.95)	5.67 (4.70–6.41)	3.10 (2.00–4.80)	5.86 (3.98–8.88)	<.001
Neutrophils (10^9/L)*	2.89 (2.40–3.54)	2.83 (2.37–3.40)	1.60 (1.00–2.40)	4.0 (2.74–6.93)	<.001
Lymphocytes (10^9/L)*	1.77 (1.49–2.06)	2.00 (1.53–2.47)	0.90 (0.59–1.37)	0.80 (0.56–1.17)	<.001
Monocytes (10^9/L)*	0.30 (0.25–0.36)	0.48 (0.40–0.67)	0.26 (0.18–0.50)	0.42 (0.30–0.60)	<.001
RDW (%)*	12.55 (12.20–13.00)	13.4 (12.8–14.2)	16.30 (14.90–18.03)	17.20 (14.50–19.50)	<.001
MPV (fL)*	8.60 (8.00–9.40)	9.00 (8.40–10.00)	9.65 (8.80–10.60)	10.00 (9.00–11.00)	<.001
RPR*	0.06 (0.05–0.07)	0.07 (0.06–0.10)	0.21 (0.14–0.39)	0.23 (0.16–0.33)	<.001
MPR*	0.04 (0.03–0.05)	0.06 (0.04–0.08)	0.13 (0.07–0.19)	0.13 (0.08–0.20)	<.001
PLR*	128.43 (101.94–154.68)	86.43 (65.75–121.72)	83.12 (56.67–111.43)	92.08 (62.76–127.50)	<.001
NLR*	1.62 (1.29–2.02)	1.53 (1.15–1.84)	1.93 (1.31–2.36)	4.84 (2.94–9.06)	<.001
MLR*	0.17 (0.14–0.21)	0.25 (0.20–0.33)	0.33 (0.22–0.43)	0.52 (0.35–0.83)	<.001
PNI*	53.50 (51.65–56.13)	49.50 (45.40–53.40)	43.80 (39.15–48.45)	33.70 (28.75–37.75)	<.001
CTP[‡]	NA	NA	5.00 (5.00–6.00)	12.00 (10.00–13.00)	<.001
MELD[‡]	NA	NA	5.26±2.69	22.11±6.90	<.001
Antiviral therapy-n (%)					
ADV[†]	0	1 (1.6)	3 (3.8)	3 (1.5)	.501
ETV[†]	0	46 (73.0)	57 (72.2)	118 (58.1)	.103
LAM[†]	0	6 (9.5)	6 (7.6)	38 (18.7)	.027
LDT[†]	0	2 (3.2)	7 (8.9)	14 (6.9)	.412
ETV/LAM/LDT+ADV[†]	0	8 (12.7)	6 (7.6)	30 (14.8)	.267

ACLF = acute-on-chronic liver failure, ALB = albumin, CHB = chronic hepatitis B subjects without cirrhosis and liver failure, Cr = creatine, CTP = Child-Turcotte-Pugh score, HS = healthy subjects, INR = international normalized ratio, LC = liver cirrhosis, MELD = model for end-stage liver disease, MLR = monocyte-to-lymphocyte ratio, MPR = MPV-to platelet ratio, MPV = mean platelet volume, NA = not available, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, PLT = platelet, PNI = prognostic nutritional index, RDW = red cell distribution width, RPR = RDW-to platelet ratio, TBIL = total bilirubin, WBC = white blood cell.

* As compared among the 4 groups.

[†] As compared among CHB, LC, and ACLF groups.

[‡] As compared between LC and ACLF groups.

41.9% (85/203). During hospitalization, 129 patients progressed to at least 1 other type of organ failure. Among them, 23, 38, 36, 23, and 118 cases progressed to hepatic encephalopathy (grade 3 or 4), renal failure, lung failure, circulation failure, and coagulation failure in ACLF patients, respectively.

3.2. Levels of inflammation-based markers in patients with HBV-related ACLF

Compared with patients in LC, CHB and HS group, WBC was significantly higher in patients with liver failure (*P*<.001). We also found that circulating neutrophil and monocyte counts were significantly higher while lymphocyte counts were lower in ACLF patients compared to those in patients in LC, CHB, and HS groups (*P*<.001) (Table 1).

Our findings also indicated that the neutrophil-to lymphocyte ratio (NLR) in HBV-ACLF patients was significantly higher compared to that in LC, CHB and HS groups. Significant positive correlations between NLR and both WBC (r=0.615, *P*<.001) and MELD scores (r=0.381, *P*<.001) were detected in ACLF patients. The MLR in ACLF patients was also significantly increased compared to that in LC, CHB, and HS. The MLR was positively correlated with WBC (r=0.405, *P*<.001) and MELD scores (r=0.274, *P*<.001) (Table 1, Fig. 1).

We also evaluated the clinical implications of RDW, mean platelet volume (MPV), RPR, MPR, PLR, and PNI. These markers can also reflect systemic inflammation. We found that the levels of RDW, MPV, RPR, and MPR were significantly higher while PNI was lower in patients with liver failure at presentation compared with the levels in LC, CHB, and HS groups (*P*<.001). In addition, the levels of PLR were higher in ACLF patients than in LC and CHB groups, but lower than that in the HS group (*P*<.001) (Table 1).

3.3. Comparison of inflammation-based markers between survivors and nonsurvivors in ACLF group

According to the survival outcome within 90-day after recruitment, patients were divided into a survival group and a nonsurvival group. We then analyzed the association between inflammatory markers and disease prognosis in patients with ACLF further. Levels of NLR and MLR were significantly higher in non-survivors than in survivors (*P*<.001) (Fig. 2). However, with respect to other inflammatory markers such as RDW, MPV, RPR, MPR, PLR, and PNI, there were no differences between survivors and nonsurvivors (*P*>.05) (Table 2).

Multivariate Cox regression analyses were performed on ACLF patients, including parameters at presentation (TBIL, Cr,
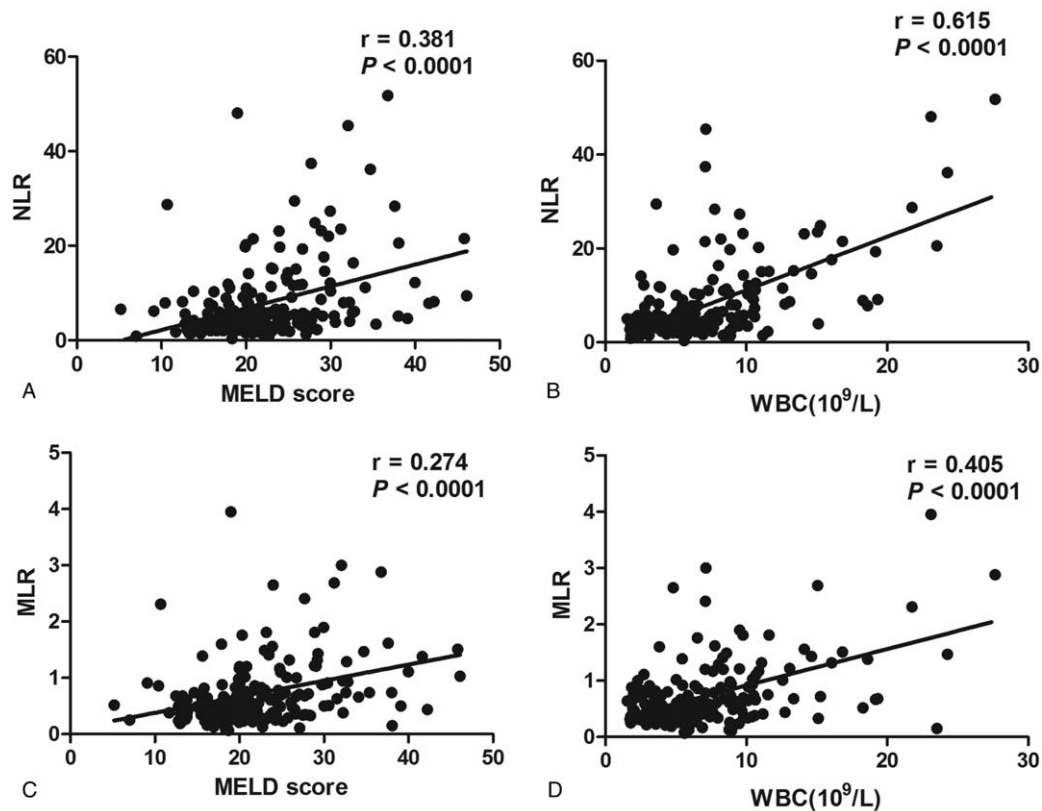
Figure 1. NLR and MLR levels on presentation correlate with MELD score and WBC in patients with HBV-related acute-on-chronic liver failure, respectively. (A) NLR and MELD; (B) NLR and WBC; (C) MLR and MELD; (D) MLR and WBC. HBV = hepatitis B virus, MELD = model for end-stage liver diseas, MLR = monocyte-to-lymphocyte ratio, NLR = neutrophil-to-lymphocyte ratio, WBC = white blood cell.

INR, WBC, neutrophil counts, monocyte counts, NLR, MLR, CTP, MELD, and CLIF-SOFA scores), where univariation was correlated to death. As a result, TBIL, NLR, CTP, and CLIF-SOFA scores were independent risk factors for prognosis. The hazard ratios (HRs) were 1.002, 1.044, 1.247, and 1.230, respectively (Table 3).

Receiver operating characteristic (ROC) curves for parameters at presentation including TBIL, NLR, MLR, CTP, MELD, and CLIF-SOFA scores are shown in Figure 3.D. The area under the ROC curve (AUC) for NLR was 0.748 [95% CI (0.679–0.816), $P < .001$], for MLR was 0.723 [95%CI (0.651–0.795), $P < .001$], for CLIF-SOFA was 0.737 [95%CI (0.665–0.808), $P < .001$], for CTP was 0.708 [95%CI (0.636–0.781), $P < .001$], for MELD was 0.714 [95%CI (0.640–0.788), $P < .001$] and for TBIL was 0.573 [95%CI (0.490–0.657), $P = .079$]. NLR, MLR, and CLIF-SOFA scores had the higher AUC for identified worse outcome than MELD, CTP, and TBIL. Because they were more accurate, user-friendly, inexpensive and reproducible, NLR and MLR were selected in combination for an improved predictive ability. We found that the combination of NLR and MLR demonstrated the highest AUC for predicting poor prognosis in ACLF patients (Table 4).

To further assess the combined impact of NLR and MLR on survival, HBV-ACLF patients were divided into 4 groups using the cut-off value: NLR≥5.09 and MLR≥0.62; NLR≥5.09 and MLR <0.62; NLR<5.09 and MLR≥0.62; NLR<5.09 and MLR<0.62. The results showed that the group with NLR≥5.09 and MLR≥0.62 (both high group) had a

significantly lower survival rate than the other 3 groups (Fig. 3C).

3.4. Dynamic changes of inflammation-based markers levels in ACLF patient

Peripheral blood samples were dynamically obtained from ACLF patients every 7 days during their hospitalization for 4 weeks. The nonsurvivor group had 73, 57, 39, 28 surviving patients on 7, 14, 21, 28 days, respectively. There was a persistent difference in TBIL, INR, monocyte counts, NLR and MLR between survivors, and nonsurvivors. The levels of TBIL, INR, monocyte counts, NLR and MLR in nonsurvivors continually fluctuated at a high level (Fig. 4).

4. Discussion

Systemic inflammatory responses (SIRS) occur frequently in patients with liver failure and are correlated with the severity of liver disease and its prognosis.[7,8] At admission, 64.2% patients with ACLF presented SIRS with or without infection. SIRS was associated to portal hypertension-related complications and death.[17] The 90-day mortality rate was 65% in patient with SIRS and 42.8% in patients without SIRS.[7] Systemic inflammation in HBV-ACLF patients was characterized by an excessive innate immune response, which was correlated with disease progression and deterioration.[18] Many studies demonstrated that inflammatory response can be reflected by the level of white
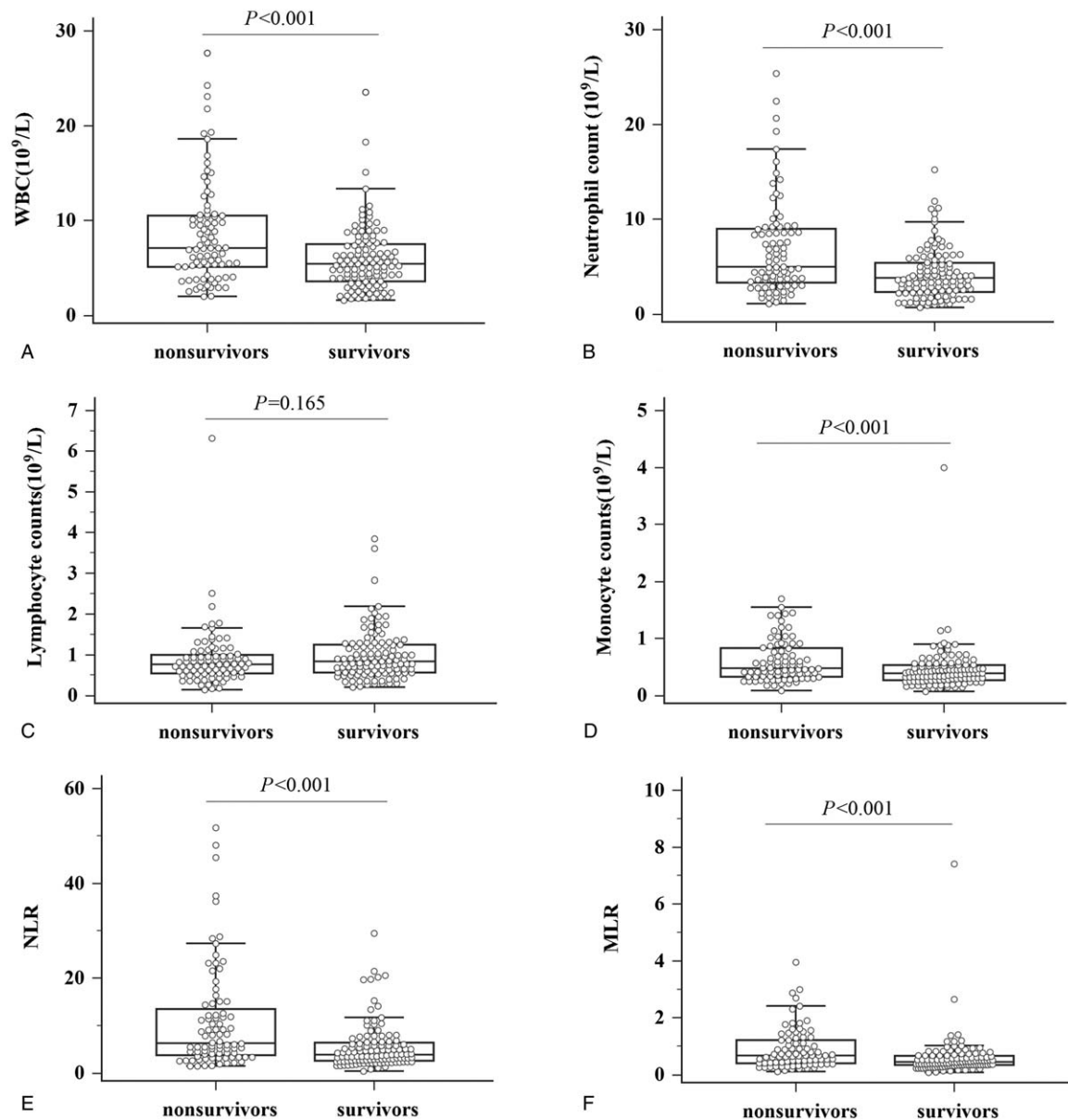
Figure 2. Comparison of WBC (A), neutrophil counts (B), lymphocyte counts (C), monocyte counts (D), NLR (E) and MLR (F) levels between nonsurvivors and survivors in patients with HBV-ACLF. HBV-ACLF = hepatitis B virus-related acute-on-chronic liver failure, MLR = monocyte-to-lymphocyte ratio, NLR = neutrophil-to-lymphocyte ratio, WBC = white blood cell.

blood cells, neutrophils, lymphocytes, platelets, and acute-phase proteins.[12–15] As a result, series of combinations of these markers such as NLR, MLR, PLR, RPR, MPR, and PNI might be potential prognostic indicators for liver failure.

In this study, we found that NLR, MLR, RDW, MPV, RPR, and MPR were increased while PNI were decreased in ACLF patients compared to those in the LC, CHB, and HS groups ($P < .001$). The relationship between inflammation and liver failure has been studied for many years. It is increasingly recognized that the SIRS plays a crucial role in development and progression of liver failure.[7,8] Acute hepatic insults such as bacterial infection, use of hepatotoxic drugs, gastrointestinal bleeding, and HBV reactivation leading to ACLF are the result of the widespread activation of the inflammatory cytokine pathways.[18,19] Patients with ACLF display "sepsis-like" immune

paralysis.[20] Studies have previously demonstrated that the levels of various serum inflammatory cytokines are significantly different between patients with ACLF and stable cirrhosis.[8,21] Unbalanced inflammatory response is closely associated with mortality in ACLF patients. Under inflammatory stress, neutrophils, lymphocytes, and monocytes interact with parenchymal and non-parenchymal cells through the secretion of cytokines and promote the progression of the disease.[7,10,11] Platelets help to and regulate inflammatory and immune responses and play an important role in hemostasis and thrombosis.[22] RDW is a measure of variation in erythrocyte volume and has been reported to be a predictor of mortality in certain disorders, including stroke and infection.[12,22] As such, these inflammation-based indicators could be useful for stratifying the severity of liver failure.

Table 2

Comparisons of characteristics between survivors and nonsurvivors in patients with HBV-ACLF.

Variables	Survivors N = 118	Non-survivors N = 85	P value
Age, years	52.50 ± 12.05	52.89 ± 11.21	.391
Gender, M:F	83/35	68/17	.120
ALB, g/L	29.50 ± 4.79	28.43 ± 5.98	.054
TBIL, μmol/L	194.80 (121.13–284.43)	225.80 (124.45–381.20)	<.001
Cr, μmol/L	58.00 (48.00–72.00)	71.00 (53.0–104.00)	.003
INR	2.02 (1.67–2.36)	2.48 (1.92–2.91)	<.001
HBV-DNA-log$_{10}$, IU/mL	5.84 (4.90–7.18)	5.70 (4.42–7.36)	.429
HBeAg positive-n, %	71 (60.1)	46 (54.1)	.389
PLT, 10^9/L	78.00 (49.00–127.25)	64.00 (53.50–105.00)	.218
WBC, 10^9/L	5.42 (3.57–7.52)	7.10 (5.12–10.55)	<.001
Neutrophils, 10^9/L	3.82 (2.35–5.42)	4.97 (3.23–9.05)	<.001
Lymphocytes, 10^9/L	0.84 (0.57–1.25)	0.77 (0.54–1.01)	.165
Monocytes, 10^9/L	0.39 (0.27–0.53)	0.48 (0.33–0.84)	.001
RDW, %	16.60 (15.05–18.55)	15.80 (14.90–17.45)	.102
MPV, fL	9.80 (8.65–10.70)	9.60 (8.90–10.55)	.628
RPR	0.21 (0.14–0.35)	0.25 (0.16–0.32)	.530
MPR	0.13 (0.08–0.20)	0.15 (0.09–0.21)	.184
PLR	87.29 (65.12–123.94)	101.01 (57.96–149.71)	.494
NLR	3.54 (2.37–5.77)	7.90 (4.34–14.95)	<.001
MLR	0.44 (0.33–0.62)	0.71 (0.45–1.31)	<.001
PNI	34.25 (30.71–38.15)	32.70 (27.68–36.93)	.063
CTP	11 (10–12)	12 (11–13)	<.001
MELD	20.02 ± 5.59	25.00 ± 7.52	<.001
CLIF-SOFA	7.00 (6.00–7.00)	8.00 (7.00–9.00)	<.001
Antiviral therapy-n (%)			
ADV	2 (1.7)	1 (1.2)	1.000
ETV	67 (56.8)	51 (60.0)	.646
LAM	22 (18.6)	16 (18.8)	.974
LDT	9 (7.6)	5 (5.9)	.628
ADV+ETV/LAM+ADV	18 (15.3)	12 (14.1)	.822

ADV = adefovir dipivoxil, ALB = albumin, CLIF-SOFA = chronic liver failure-sequential organ failure assessment score, Cr = creatine, CTP = Child-Turcotte-Pugh score, ETV = entecavir, INR = international normalized ratio, LAM = lamivudine, LDT = telbivudine, MELD = model for end-stage liver disease, MLR = monocyte-to-lymphocyte ratio, MPR = MPV-to platelet ratio, MPV = mean platelet volume, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, PLT = platelet, PNI = prognostic nutritional index, RDW = red cell distribution width, RPR = RDW-to platelet ratio, TBIL = total bilirubin, WBC = white blood cell.

Table 3

Cox regression analysis for variables associated with 90-day mortality in patients with HBV-ACLF.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age	1.018	0.999–1.038	.060			
Gender	0.666	0.391–1.132	.133			
ALB, g/L	0.974	0.934–1.017	.235			
TBIL, μmol/L	1.003	1.001–1.004	<.001	1.002	1.001–1.004	.002
Cr, μmol/L	1.008	1.004–1.012	<.001			
INR	1.225	1.099–1.365	<.001			
PLT, 10^9/L	0.997	0.992–1.001	.171			
WBC, 10^9/L	1.098	1.058–1.139	<.001			
Neutrophils, 10^9/L	1.123	1.077–1.171	<.001			
Lymphocytes, 10^9/L	0.821	0.547–1.233	.343			
Monocytes, 10^9/L	1.555	1.127–2.146	.007			
RDW, %	0.932	0.849–1.024	.142			
MPV, fL	1.029	0.902–1.173	.672			
RPR	1.304	0.394–4.315	.663			
MPR	2.220	0.324–15.235	.417			
PLR	1.003	0.999–1.006	.138			
NLR	1.056	1.036–1.076	<.001	1.044	1.023–1.066	<.001
MLR	1.731	1.466–2.043	<.001			
PNI	0.977	0.945–1.011	.187			
CTP	1.418	1.226–1.639	<.001	1.247	1.073–1.451	.004
MELD	1.097	1.066–1.128	<.001			
CLIF-SOFA	1.326	1.230–1.429	<.001	1.230	1.115–1.357	<.001

ALB = albumin, CLIF-SOFA = chronic liver failure-sequential organ failure assessment score, Cr = creatine, CTP = Child-Turcotte-Pugh score, INR = international normalized ratio, MELD = model for end-stage liver disease, MLR = monocyte-to-lymphocyte ratio, MPR = MPV-to platelet ratio, MPV = mean platelet volume, NLR = neutrophil-to-lymphocyte ratio, PLR = platelet-to-lymphocyte ratio, PLT = platelet, PNI = prognostic nutritional index, RDW = red cell distribution width, RPR = RDW-to platelet ratio, TBIL = total bilirubin, WBC = white blood cell.

The existence of inflammation is believed to be pathogenic in the development of an impaired nutritional status.[14] As a negative acute phase response reactant, serum albumin could reflect the nutritional status. With the deterioration of liver function, albumin synthesis is reduced. Hypoalbuminemia is a common complication in patients with liver cirrhosis which can cause ascites and edema, even tendency of spontaneous bacterial peritonitis and accounted for increased mortality.[23] Albumin<25 g/L is an independent predictor for poor outcome in patients with acute pancreatitis.[24] Furthermore, the PNI which includes 2 components (serum albumin and lymphocyte counts) is also an independent predictor of poor prognosis in patients with HCC.[14] As such, clinicians may stratify the severity of disease in patients with ACLF based on PNI, reflecting both the presence of SIRS and the progression of malnutrition.

Notably, Levels of NLR and MLR were significantly higher in non-survivors than in survivors (P<.001). However, with respect to other inflammatory markers such as RDW, MPV, RPR, MPR, PLR, and PNI, there was no difference between survivors and non-survivors (P>.05). NLR is a promising parameter that reflects systemic inflammation and the general nutritional status of patients. Recently, an accumulation of evidence points to elevated NLR is an independent predictor of poor prognosis in patients with decompensated cirrhosis and hepatocellular carcinoma.[13,14] The complicated physiopathologic association between an elevated NLR and a poor prognosis remains unclear. The widely accepted hypothesis is that elevated NLR reflects the severity of the potentially acute systemic inflammation following primary injury.

Neutrophils and lymphocytes are 2 major cell components of the immune system. Neutrophils reflect any ongoing inflammation, while lymphocyte counts represent the immunomodulatory pathway. Neutrophils promote inflammation via the secretion of a series of inflammatory cytokines (IL-1, IL-8) and releasing granule-containing enzymes such as myeloperoxidase, elastase and collagenase.[25] Myeloperoxidase can directly cause tissue damage and produces hypochlorous acid from hydrogen peroxide and chloride anions during neutrophil oxidative respiratory bursts. This process is known to cause oxidative damage in tissue.[26] Neutrophil elastase may be involved in neutrophil-induced IL-1β maturation, leading to an inflammation cascades and cause tissue damage by direct cytotoxicity to cells and degradation the extracellular matrix and basement membrane.[26,27] Recently, it was reported that neutrophils mediate insulin resistance via secreted elastase.[27] Moreover, in patients with liver failure, circulating neutrophils have exhibited impaired phagocytic functions and bactericidal capacities. Intestinal bacterial overgrowth, or dysbiosis, increases intestinal permeability and immunodeficiency and results in bacterial translocation. The impaired phagocytic functions of immune cells in conjunction with portosystemic shunting increase endotoxin in circulation. Endotoxins activate the immune system and promote the release of proinflammatory cytokines. These inflammatory mediators lead to the development of SIRS and the progression to multisystem organ failure.

Lymphocytes are the principal component of the adaptive immune system and play a role in regulating subsequent systemic inflammation as the disease progress. Systemic inflammation in HBV-ACLF is the result of depletion in circulating lymphocytes.[28] Increasingly data have shown that lymphopenia might be a predictor for poor prognosis in patients with acute pancreatitis, malignant diseases, and chronic liver disease.[12–15] Under uncontrolled inflammation, reduced peripheral lymphocytes may result from
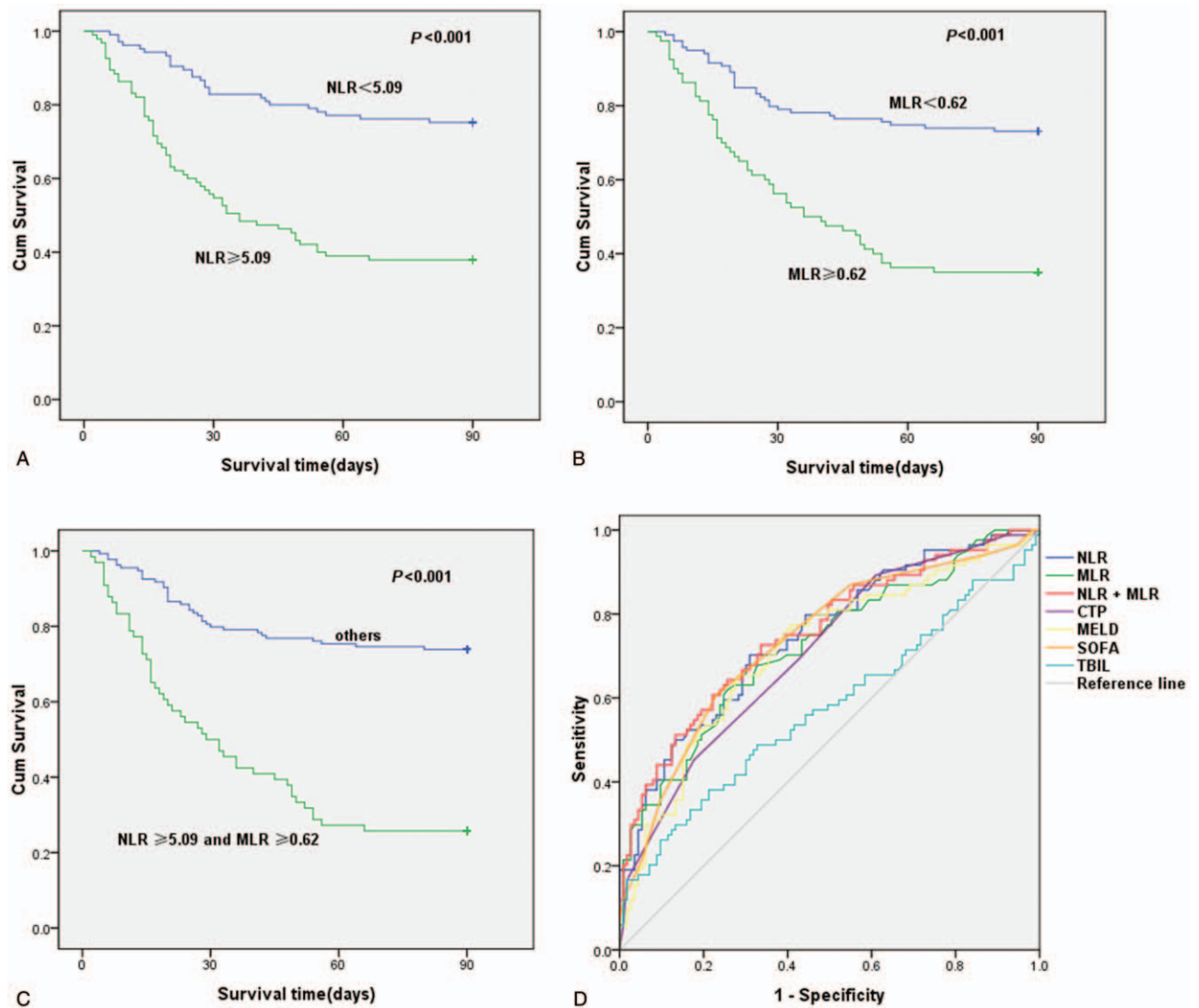
Figure 3. Kaplan–Meier survival curves in patients (A) patients with NLR≥5.09 and NLR <5.09 (B) patients with MLR≥0.62 and MLR <0.62 (C) patients with both NLR≥5.09 and MLR≥0.62 and others. D. Receiver operating characteristics (ROC) curve analysis for prediction of 90-day mortality by NLR, MLR, NLR combined with MLR, TBIL, CTP, MELD, and CLIF-SOFA. CLIF-SOFA=chronic liver failure-sequential organ failure assessment, CTP=Child-Turcotte-Pugh, MELD=model for end-stage liver diseas, MLR=monocyte-to-lymphocyte ratio, NLR=neutrophil-to-lymphocyte ratio, TBIL=total bilirubin.

cell apoptosis, necrosis, and redistribution. For example, mostly peripheral CD4+T cells are naïve lymphocytes. Activated and differentiated CD4+ T cells are recruited into the inflamed liver, where they contribute to hepatic inflammation.[28] Intrahepatic CD8 + T-cell numbers are approximately 50-fold greater in ACLF patients than in normal individuals.[29] The excessive release of cytokines by CD4+ and CD8+ T lymphocytes promotes inflammatory reactions that can lead to massive liver damage.

Table 4

Receiver operating characteristics curve of prognostic variables for patients with HBV-ACLF.

Variable	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
NLR	5.09	0.748 (0.679–0.816)	0.702	0.690	0.621	0.759	<.001
MLR	0.62	0.723 (0.651–0.795)	0.619	0.743	0.642	0.729	<.001
NLR+MLR		0.755 (0.686–0.823)	0.726	0.667	0.616	0.768	<.001
CTP	10.50	0.708 (0.636–0.781)	0.894	0.389	0.507	0.830	<.001
MELD	21.92	0.714 (0.640–0.788)	0.655	0.717	0.625	0.746	<.001
CLIF-SOFA	7.50	0.737 (0.665–0.808)	0.607	0.770	0.654	0.727	<.001
TBIL, μmol/L	298.90	0.573 (0.490–0.657)	0.381	0.788	0.561	0.641	.079

CLIF-SOFA=chronic liver failure-sequential organ failure assessment score, CTP=Child-Turcotte-Pugh score, MELD=model for end-stage liver disease, MLR=monocyte-to-lymphocyte ratio, NLR= neutrophil-to-lymphocyte ratio, NPV=negative predictive value, PPV=positive predictive value, TBIL=total bilirubin.
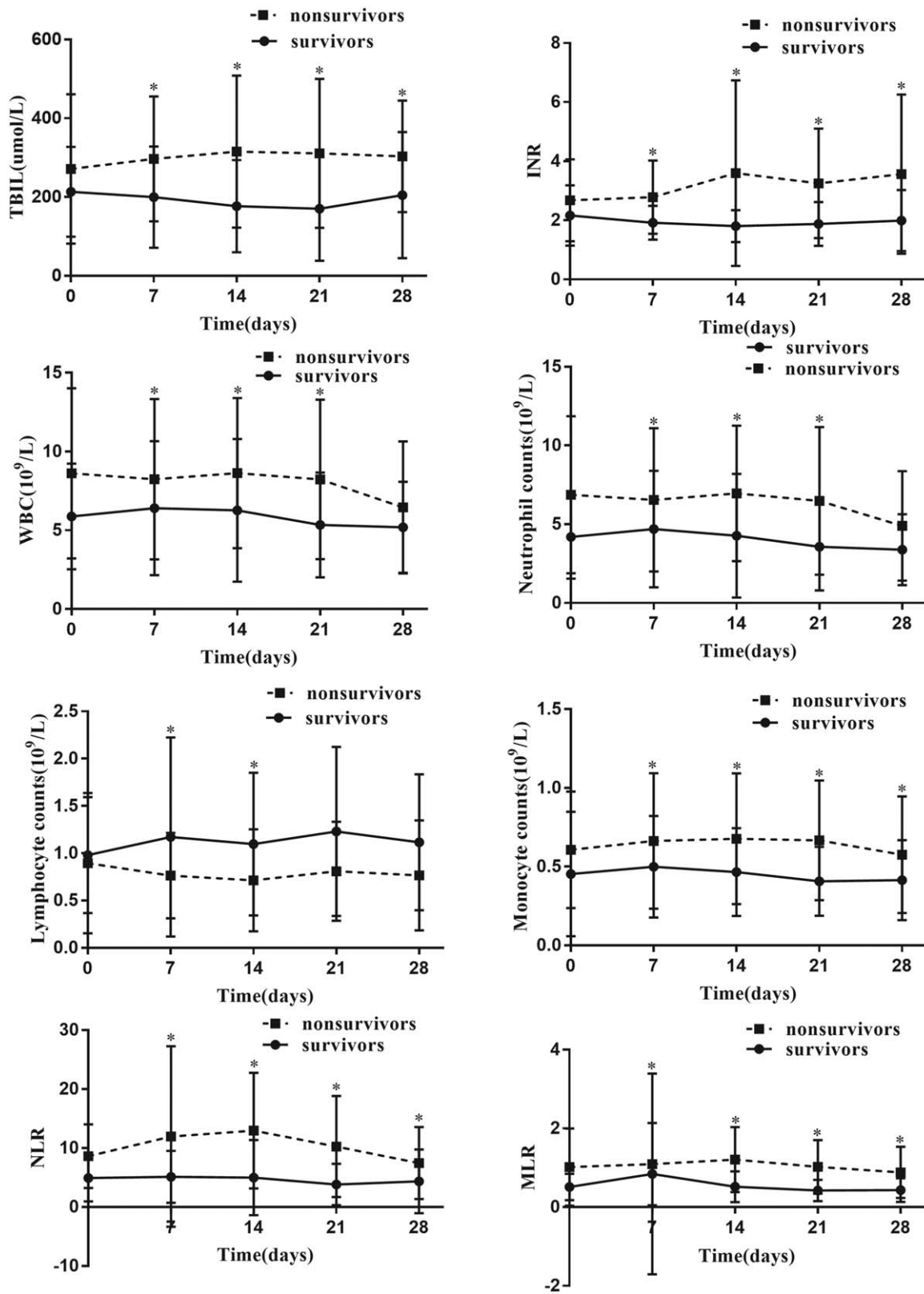
Figure 4. Dynamic changes of the WBC, neutrophils, lymphocytes, monocytes, NLR and MLR levels in survivors and nonsurvivors in ACLF patients. $^{*}P <.05$ for survivors compared with nonsurvivors. ACLF = acute-on-chronic liver failure, MLR = monocyte-to-lymphocyte ratio, NLR = neutrophil-to-lymphocyte ratio, WBC = white blood cell.

The elevated levels of MLR in ACLF patients primarily results from an increased number of monocytes and a decreased number of lymphocytes compared to that in other groups. The inflammatory response can trigger the release of monocytes from the bone marrow to the peripheral blood and the differentiation of peripheral monocytes into tissue macrophages.[30] Intrahepatic infiltrated monocytes represent an important constitution of the innate immune response. Under pathological stress, such as steatohepatitis and viral hepatitis, Kupffer cells can differentiate from infiltrated bone marrow-derived monocytes to clear debris and microbial pathogens and to support the restoration of the tissues to a pre-inflammatory state. Kupffer cells can be constantly replenished by blood monocytes.[31] In addition, Kupffer cells can perpetuate inflammation by releasing pro-inflammatory cytokines, including TNF-a, IL-1β, IL-6, and IL-8.[31] Excessive inflammatory cytokines can trigger the release of monocytes from the bone marrow which migrates into the inflamed liver, forming a vicious circle. A disturbed peripheral immune response is associated with organ failure and death. Therefore, the ratio of monocytes to lymphocytes provides an indicator for reflecting ongoing inflammation that may lead to organ failure.

Nevertheless, many limitations should be taken into consideration. First, this study was a single-center, observational study performed on patients at the Tianjin Third Central Hospital with a small sample size. Therefore, there was a selective bias and causal relationships between these markers and all-cause mortality cannot be established. Second, our study did not assess other pro-inflammatory cytokines, such as TNF-a, IL-1β, IL-6, and IL-8, that might be helpful in establishing a mechanism.

In conclusion, our study demonstrated that elevated levels of inflammatory markers can be used to determine the severity of liver disease. NLR and MLR were found to be associated with risk of death while NLR was an independent predictor of poor outcome in ACLF patients. The combination of NLR and MLR is more accurate for predicting poor outcome than either marker alone in ACLF patients. This combination is superior to the CLIF-SOFA, MELD, CTP score, and TBIL in terms of prognostic ability. Furthermore, both NLR and MLR are accurate, user-friendly, inexpensive and reproducible markers for ACLF prognosis and monitoring. In future, multiple-center, randomized, prospective studies involving a larger sample size would be necessary to evaluate the association between these 2 parameters and ACLF.

Author contributions

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Project administration: Huiqing Jiang.
Resources: Tao Han.
Software: Kai Wang.
Writing – original draft: Junjun Cai, Tao Han.
Writing – review & editing: Kai Wang, Huiqing Jiang.

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