International Immunopharmacology 56 (2018) 58–64

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Prognostic nomogram incorporating neutrophil-to-lymphocyte ratio for early mortality in decompensated liver cirrhosis

Lin Lin[1], Fang Yang[1], Ya Wang, Shuai Su, Zhengyan Su, Xihui Jiang, Yanmin Zheng, You Deng, Houning Lv, Jingwen Zhao, Rui Lin, Bangmao Wang, Chao Sun*

Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital, Anshan Road 154, Heping District, Tianjin 300052, China
Tianjin Institute of Digestive Disease, Tianjin Medical University General Hospital, Anshan Road 154, Heping District, Tianjin 300052, China

ARTICLE INFO

Keywords:
Neutrophil-to-lymphocyte ratio
Prognosis
Chronic liver disease
Cytokine

ABSTRACT

Background: Neutrophil-to-lymphocyte ratio (NLR) is a marker of systemic inflammation. However, its predictive utility of 30-day mortality remains elusive in decompensated cirrhotics.
Aims: We aimed to combine NLR and other variables associated with early mortality of cirrhotics with acute insults in to a predictive nomogram.
Methods: We retrospectively analyzed 352 decompensated cirrhotics. The 30-day mortality was regarded as primary outcome. Multivariate Cox analysis was performed, and a NLR-based nomogram was developed. The performance of nomogram was determined in terms of its calibration, discrimination and clinical usefulness. Serum cytokines were evaluated by Milliplex cytokine assay.
Results: On multiple analysis, independent factors for early mortality were albumin, MELD and NLR, which were all selected into the nomogram. The nomogram showed good discrimination, with a concordance index of 0.88. Calibration of the nomogram predicted survival corresponding optimally with the actual outcomes. Decision curve analysis indicated our nomogram was useful in clinical practice. Among circulating cytokines we investigated, IL-6 and IL-8 were substantially elevated in cirrhotics compared to healthy subjects. High NLR was positively correlated with the expression of IL-6 and IL-8.
Conclusion: The proposed nomogram incorporating NLR offered an individualized predictive tool for 30-day mortality in decompensated cirrhotics. The escalating value of NLR likely implicated excessive inflammatory response.

1. Introduction

The course of liver cirrhosis (LC) is complicated by two concurrent and interlinked detriments, systemic inflammation and immunodeficiency [1]. Inflammation is indicative of an increased production of pro-inflammatory cytokines and their elevated circulating levels. Immunodeficiency stems from damages to the immune response at both hepatic and systemic levels. These dynamic alterations may account for pathophysiological basis for various clinical manifestations in cirrhotics. For instance, the influenced spectrum spans from vulnerable to bacterial infection, to organ-specific dysfunction as well as concomitant acute insults [2,3]. However, both Child-Turcotte-Pugh (CTP) and model for end-stage liver disease (MELD) classification, two well established scores, have omitted the immune dysregulation which is thought to be highly concerned with the prognosis in decompensated LC [4]. Collectively, it is imminent to incorporate indicator of distorted immune response to prognostication system.

Neutrophil-to-lymphocyte ratio (NLR) represents the imbalance of two distinct immune pathways [5,6]. The neutrophil count refers to ongoing inflammation, whilst the lymphocyte count refers to the regulatory immune pathway [7]. Moreover, the predictive usefulness of escalating NLR has now been extensively investigated in myriad hepatic entities, taking account of adverse outcomes, advanced histologic stage or poor treatment response [8,9]. More recently, raised NLR was shown to predict medium- and long-term mortality in decompensated LC without acute-on-chronic liver failure (ACLF) [7]. Despite this, it

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; LC, liver cirrhosis; MELD, model for end-stage liver disease; CTP, Child-Turcotte-Pugh classification; ACLF, acute-on-chronic liver failure; LMR, lymphocyte-to-monocyte ratio; INR, international normalized ratio; Scr, serum creatinine; C-index, concordance index; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CI, confidence interval

* Corresponding author.
E-mail address: chaosun@tmu.edu.cn (C. Sun).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.intimp.2018.01.007
Received 14 September 2017; Received in revised form 7 December 2017; Accepted 4 January 2018

remains elusive whether elevated NLR might identify early mortality at 30 days.

Nomogram is widely applied as a statistical prognostic model in medicine [10]. It results in an individual probability of a clinical event, such as death or recurrence, by combining diverse biological and clinical variables. Medical nomograms are beneficial for personalized decision making during daily practical encounters [11]. Recently, some investigators have introduced nomograms for evaluating the outcomes in liver diseases [12,13].

Therefore the purpose of present study was: (1) to construct a clinically useful nomogram with full consideration of hepatic and immune dysfunction; (2) to validate the nomogram by internal set; and (3) to investigate the associations between NLR and several serum cytokines in LC subjects.

2. Materials and methods

2.1. Study population

Patients were consecutively recruited from Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital between February 2014 and February 2017. The inclusion criteria were as follows: (1) patients aged over 18 years; (2) diagnosis of LC (on account of clinical, laboratory, imaging examinations, transient elastography results or biopsy confirmations; and (3) presence of acute decompensated events within previous 2 weeks, including gastroesophageal varices hemorrhage, development of large ascites (grades 2 and 3 according to the international ascites club classification; first or new onset of ascites), hepatic encephalopathy (HE, acute changes in mental status without evidence of neurological disease), infections (combination of clinical, laboratory indictors or positive microbial detection), hepatorenal syndrome (HRS) or any combination of these [14]. Exclusion criteria were: (1) presence with ACLF at admission (APASL definition) [15]; (2) primary liver carcinoma or other malignant tumors with or without metastasis; (3) concurrent pregnancy; (4) immune-suppressive medication; (5) lost to follow-up or incomplete data; and (6) liver transplantation. Four hundred forty-nine LC subjects fulfilled the inclusion criteria at initial assessment, 12, 38, 19, 26 and 2 cases were excluded owing to admission ACLF, malignancies, ongoing immune-suppressive therapy, lost to follow-up or incomplete clinical parameter and liver transplantation, respectively. Finally, a total of 352 decompensated LC patients were left for final analysis. This study was conducted in accordance with the Declaration of Helsinki and was approved by Ethics Committee of Tianjin Medical University General Hospital. Two hundred and thirty-five cases who were enrolled between February 2014 and July 2016 were assigned to the derivation cohort, and all other 117 patients were regarded as the validation cohort (approximate ratio at 2:1).

2.2. Data collection

We retrieved demographic information, clinical features and laboratory parameters, including age, sex, etiology of LC, presence of acute insults, complete blood count, hepatic function tests, coagulation examinations, electrolytes and serum creatinine (Scr) for each enrollment in detail. All baseline laboratory tests were performed within 24 h after admission. The primary outcome was defined as deceased of 30-day follow-up duration. The complete blood count was measured by a Sysmex XE-2100 automated hematology analyzer (Sysmex Corp., Kobe, Japan). The NLR was calculated by dividing the absolute neutrophil count by absolute lymphocyte count, according to the differential white blood cell count [16]. MELD score was calculated as following: $MELD = 9.6 \times \ln$ [Scr (mg/dl)] $+ 3.8 \times \ln$[Total bilirubin (mg/dl)] $+ 11.2 \times \ln$[Prothrombin time (INR)] $+ 6.4$ [17].

2.3. Serum samples collection and analysis

Circulating cytokine profiles encompassed subjects from the validation cohort who gave information consent (available in ninety-eight cases). For the cytokine assays, whole blood samples were collected into disposable vacuum blood collection tubes (BD, USA). After 0.5 h of standing in room temperature, and centrifuged at 2000 rpm/min for 10 min; serum was then obtained. The supernatant was pipetted in to EP tubes and stored at −80 °C until use. We quantitatively detected the expression level of seven circulating cytokines, including IL-1β, IL-6, IL-8, IL-10, IL-17A, TNF-α and IFN-γ using MILLIPLEX® map Human High Sensitivity Cytokine Panels for 96-well assay (Millpore Corporation, Billerica MA, USA) on a Luminex platform [18]. Only measurements with CV ≤ 20% were included in the analysis. All cytokine concentrations were analyzed in the same bead suspension to minimize inter-experimental variability. For quality assurance, each sample was run twice, and the mean derivation was used as the index value.

2.4. Statistical analysis

Data were demonstrated as mean ± standard deviation or simple number as appropriate. Continuously data were compared using an independent Student *t*-test or the Mann–Whitney test for groups without normal distribution. Categorical variables were compared by chi-square test or Fisher's exact test as appropriate. Multiple comparisons were performed by using Kruskal-Wallis test with Dunn's *post hoc* test. Correlations were evaluated by the Spearman's correlation coefficient [rho (r)]. Multivariate analysis performed by Cox proportional hazard analysis was used to identify the independent parameters for 30-day mortality of patients with decompensated LC. Hazard ratio (HR) and 95% confidence interval (CI) were calculated. In circumstance, the NLR and cytokine expression levels were log2-transformed to render the data set symmetric. We considered $p < .05$ as statistically significant. SPSS (Version 21.0; IBM, New York NY, USA) and Graphpad Prism 6.01 (La Jolla, CA, USA) software are used for statistical analysis.

A nomogram based on the results of previous multivariate analysis was established by the usage of R version 3.3.2 (http://www.r-project.org/). Harrell's concordance index (C-index) was used to measure the performance of the nomogram, the larger the C-index, the more accurate was the prognostication ability of the nomogram. The calibration curve was used to analyze the agreement between nomogram and ideal observation both in the derivation and validation cohorts. The decision curve analysis was conducted to assess the clinical usefulness of the predictive nomogram by quantifying the net benefits at different threshold probabilities in both cohorts. The packages of rms, Hmisc were involved in this process.

3. Results

3.1. Baseline characteristics of hospitalized cirrhotics

Two-hundred thirty-five consecutive patients in the derivation cohort and 117 subjects in the validation cohort were enrolled for final analysis, who met the inclusive and exclusive criteria (Supplementary Fig. 1). The baseline characteristics of both cohorts were shown in Supplementary Table 1. There were 204 males (58.0%) and mean age of the study population was 60.0 ± 12.4 years. In total, twenty-five of 352 patients (7.1%) expired on 30-day follow-up. The causes of death were all attributed to cirrhosis and related complications, including liver failure (N = 10), severe infection (N = 5), esophagogastric variceal hemorrhage (N = 3), HRS (N = 3) and HE (N = 4). The etiology of LC was attributed to HBV/HCV infection in 181 (51.4%), autoimmune/cholestatic liver disease in 61 (17.3%), alcoholism in 43 (12.2%), NASH in 23 (6.5%), cryptogenic in 33 (9.4%) and other reasons in 11 (3.1%) participants. The decompensated events were precipitated by ascites in 160, gastrointestinal hemorrhage in 141,

Table 1
Univariate and multivariate analysis for 30-day mortality in derivation cohort.

Baseline parameters	Univariate analysis			Multivariate analysis[b]		
	Survival group	Deceased group	*p*-Value[a]	HR	95% CI	*p*-Value[c]
Number	218	17				
Age (years)	59.9 ± 11.8	67.0 ± 12.1	0.02			
Male (%)	121 (55.5)	12 (70.6)	0.3			
Leucocyte (10^9/L)	4.7 ± 3.3	9.7 ± 6.9	0.001			
NLR	3.9 ± 3.7	15.1 ± 13.3	< 0.001	1.108	1.065–1.154	< 0.001
LMR	2.9 ± 5.8	2.4 ± 2.6	0.02			
Hemoglobin (g/L)	95.7 ± 24.4	83.8 ± 25.3	0.05			
Platelet (10^9/L)	86.9 ± 58.5	82.0 ± 60.9	0.7			
Albumin (g/L)	30.9 ± 5.7	25.1 ± 6.4	< 0.001	0.889	0.810–0.977	0.01
ALT (U/L)	36.9 ± 52.8	54.4 ± 52.9	0.2			
AST (U/L)	51.3 ± 59.5	99.7 ± 104.7	0.2			
ALP (U/L)	109.6 ± 65.7	125.6 ± 81.0	0.4			
GGT (U/L)	104.2 ± 129.6	125.0 ± 125.8	0.6			
Total bilirubin (μmol/L)	37.1 ± 44.6	106.6 ± 121.1	0.005			
Serum creatinine (μmol/L)	68.9 ± 30.9	136.5 ± 94.6	0.003			
Sodium (mmol/L)	137.8 ± 13.9	138.9 ± 9.6	0.8			
Potassium (mmol/L)	3.8 ± 0.6	4.0 ± 0.7	0.2			
Prothrombin (INR)	1.3 ± 0.2	1.7 ± 0.7	0.04			
MELD	11.4 ± 4.1	20.1 ± 9.1	< 0.001	1.114	1.052–1.181	< 0.001[d]
CTP, A/B/C, n (%)	77 (35.3)/111 (50.9)/30 (13.8)	1 (5.9)/9 (52.9)/7 (41.2)	0.003			

Abbreviations: NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; INR, international normalized ratio; MELD, model for end-stage liver disease; CTP, Child-Turcotte-Pugh classification.
[a] *p*-Values derived from independent Student *t*-test or the Mann–Whitney test as appropriated whilst performing univariate analysis.
[b] Multivariate model includes significant variables from univariate regression in a stepwise backward analysis ($p < .05$).
[c] *p*-Values in Cox proportional hazard analysis are indicated.
[d] *p*-Value in Cox hazard regression, when treated as a continuous variable.

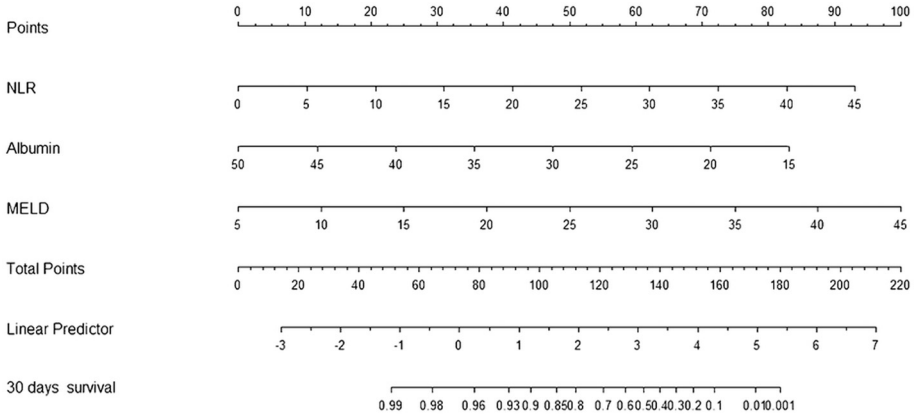

Fig. 1. Nomogram predicting 30-day overall survival in decompensated LC patients with acute insults at admission. To use the nomogram, an individual subject's value is located on each value axis, and a line is drawn upward to identify the number of points retrieved for selective variable value. The total points projected on the bottom scales indicate the probability of 30 days survival. NLR, neutrophil-to-lymphocyte ratio; MELD, model for end-stage liver disease.

infection in 23, HE in 54 and HRS in 10 patients, respectively. The mean MELD at admission was 12 ± 5.1 and 11.9 ± 5.1 in the derivation and validation cohort, respectively. The enrollment population were stratified according to CTP A/B/C in 121/180/51 subjects, respectively.

3.2. Independent prognostication for 30-day mortality

After examination of variables with significant difference by univariate analysis, age ($p = .02$), leukocyte ($p = .001$), NLR ($p < .001$), lymphocyte-to-monocyte ratio (LMR, $p = .02$), albumin ($p < .001$), Total bilirubin ($p = .005$), Scr ($p = .003$), prothrombin time (INR) ($p = .04$), MELD ($p < .001$) and CTP ($p = .003$) were fit to a multiple Cox regression model (Table 1). The hazards ratio were significantly higher for raised NLR and aggravating MELD, but lower for decreased albumin. Other variables were not found to be of independent significance. Additionally, a weakly positive correlation was observed between NLR and MELD score (r = 0.19, 95% CI 0.06–0.31, $p = .003$) (Supplementary Fig. 2).

3.3. Association of NLR and ACLF grades

More recently, an outperformed criteria for identifying ACLF was developed by Moreau et al. (EASL definition) [3]. As this definition has not been generalized across the Orient, we therefore just stratified our LC participants according to ACLF grading without incorporating it in our predictive nomogram. ACLF Grade 1 was seen in 11 patients (4.7%), Grade 2 in 11 patients (4.7%), Grade 3 in 7 patients (3.0%) and 206 patients (87.7%) were identified as ACLF Grade 0 during hospitalization. A positive association was seen in terms of NLR expression, when classified by ACLF system (r = 0.33, 95% CI 0.20–0.44, $p < .001$). The NLR was significantly decreased in patients with ACLF Grade 0 compared with that in other ACLF gradings ($p < .001$, Supplementary Fig. 2).

3.4. Construction of prognostic nomogram

Based on the results from multivariable Cox regression analyses, a nomogram was developed to predict the 30-day mortality among LC participants with acute events (Fig. 1). Specially, the nomogram was

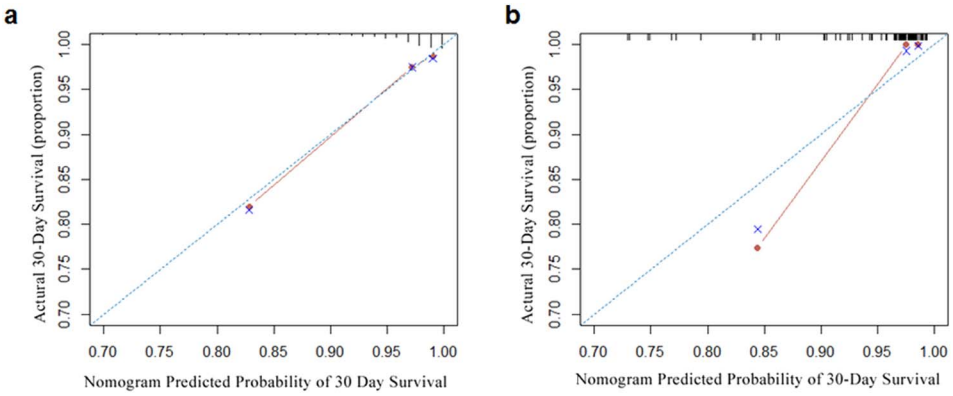

Fig. 2. The calibration curve for predicting cirrhotics survival at 30 days in the derivation cohort (a) and validation cohort (b). Nomogram-predicted probability of overall survival is plotted on the *x*-axis, actually observed overall survival (estimated by Kaplan–Meier analysis) is plotted on the *y*-axis.

generated by assigning a weighed score to each of the three independent prognostic parameters, including albumin, NLR and MELD. A higher score calculated from the sum of the assigned number of points for each predictive indicator in the nomogram correspond to a higher likelihood of decease. For instance, a LC patient with a NLR of 15 (31 points), albumin of 30 (47.5 points), MELD of 20 (37.5 points) would score a total of 116 points (and therefore have a 75% predicted risk of survival on 30-day follow-up). Participants were subsequently categorized into tertile in terms of predicted probabilities of short-term survival. Of note, patients with the highest total score (group C) were more likely to die than patients with the lowest in tertile A (86.8 *versus* 99.9% survival; $p < .001$) (Supplementary Fig. 3).

3.5. Performance of model and clinical usefulness

The discriminative ability of our final model was assessed using the C-index. In the derivation cohort, the C-index for 30-day survival prediction was 0.88 (95% CI 0.78–0.98), showing superior predictive performance to that of NLR (0.86, 95% CI 0.78–0.94) or MELD (0.84, 95% CI 0.73–0.95). Fig. 2 demonstrated the calibration curve of the nomogram. The *x*-axis represents the predicted survival resulting from the nomogram, and the *y*-axis represents actual survival estimated by the Kaplan–Meier method. The calibration plot unraveled adequate fit of the nomogram predicting the risk of death. The decision curve analysis showed that, across the entire range of threshold probability, using the nomogram to predict 30-day survival adds more net benefit in clinical decision-making (Fig. 3).

3.6. Internal validation of prognostic nomogram

Using the subsequent cohort of 117 consecutive subjects with LC from the same institution, the established nomogram gave rise to a C-index of 0.89 (95% CI 0.84–0.95) for predicting 30-day survival. Accordingly, the promising performance of the nomogram was also identified by calibration curve as well as decision curve analysis (Figs. 2 & 3).

3.7. Association of circulating cytokines and NLR

Next, in order to determine whether NLR recapitulated the prognostic information regarding the cytokine profile, we assessed the expression of several pro- and anti-inflammatory cytokines in both health controls and cirrhotics in the validation cohort. Among seven cytokines we detected, IL-6 and IL-8 expressed at significantly higher levels in LC patients who presented with acute hepatic deterioration (Fig. 4). However, no significant differences were observed on account of the expression level of serum IL-1β, IL-10, IL-17A, TNF-α or IFN-γ (Supplementary Fig. 4). A moderately positive correlation was also exhibited between increasing IL-6 and IL-8 and high NLR (Fig. 4). (See Fig. 5.)

4. Discussion

In the present study, we constructed a nomogram incorporating NLR, for the first time, to predict 30-day mortality in LC patients on acute hepatic deterioration. The proposed nomogram derived from routine laboratory data so as to maximize its clinical applicability and generality. Intriguingly, some investigators have already focused on the medium-term predictive utility of cellular makers of inflammation (such as NLR or LMR) and showed good performance of relevant nomograms, taking account of intensified management [7]. In 2015, Kwon et al. also implied that NLR was an independent factor for one-month survival among CTP class C participants [19]. Collectively, we addressed several important observations in the current study. First, the NLR positively correlated with aggravating MELD and ACLF. The higher the NLR, the greater was the probability of death. We and others

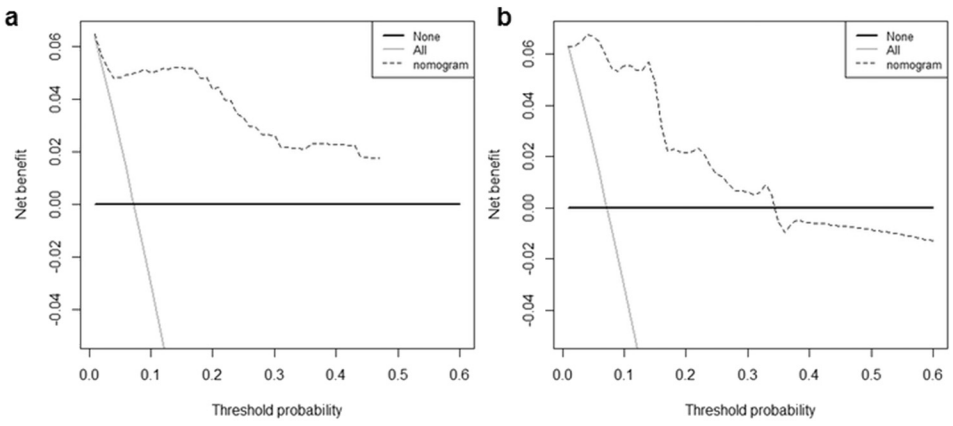

Fig. 3. Decision curve for the derivation cohort (a) and validation cohort (b) implicating the net benefit with respect to the use of the nomogram for predicting 30-day mortality in decompensated cirrhotics with acute events. Solid bold line demonstrated net benefit of treating no patients. Solid thin line demonstrated net benefit of a strategy of treating all participants. Dashed line demonstrated net benefit of a strategy of treating patients in terms of the predictive nomogram.

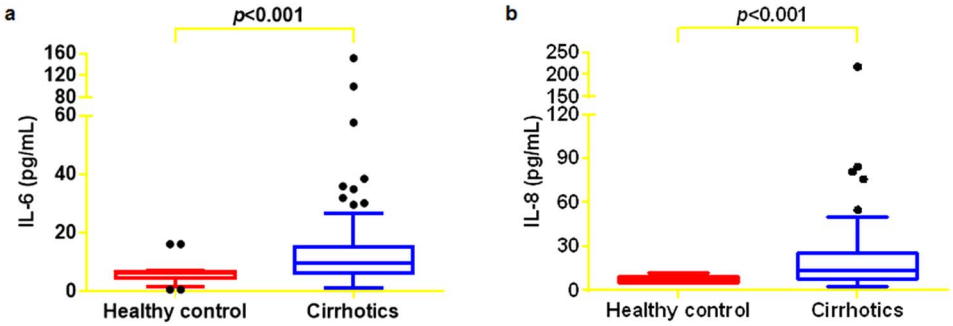

Fig. 4. Circulating IL-6 and IL-8 levels in healthy controls and decompensated cirrhotics with acute insults. IL-6 and IL-8 serum concentrations were significantly higher ($p < .001$ for both cytokines) in patients with decompensated liver cirrhosis on acute deterioration (n = 98) than healthy controls (n = 24). Tukey's box plots were shown and *p* values from Mann–Whitney test were indicated.

revealed that NLR was a valid and reliable indicator to predict mortality in cirrhotics at variable stages. Second, we provided an appreciated nomogram, which incorporating continuous variables, to assist physicians in estimating individual risk on the basis of objective parameters. Finally, our results indicated a link between the magnitude of immune distortion, mainly manifested by the expression levels of serum IL-6 and IL-8, and cellular markers of inflammation – like NLR – in LC patients.

Traditionally, CTP and MELD scores are mainstays to predict adverse outcomes regarding all aspects in LC. However, CTP encompasses a narrow range of disease severity and elusive criteria such as ascites and HE. MELD represents less prognostication accuracy in circumstance, probably due to lack of other key determinants involved in cirrhotic pathophysiology [20]. Recently, a distinctive feature, referring to cirrhosis-associated immune dysfunction syndrome (CAID), has been thoroughly described as being pivotal to the prognosis of deleterious inflammatory response in cirrhotics [1]. This syndrome refers to the combination of exacerbated inflammation and immune deficiency coexisting in the course of cirrhosis and correlating to prognosis. In the light of this, accumulative literature have sought for surrogate serum markers.

The NLR has been proposed as one such robust indictor. Most recently, Kalra et al. showed NLR closely related to liver-related death even in LC patients with low MELD [20]. Subsequently, they unveiled increased NLR corresponded to higher frequency of circulating low-density granulocytes, which displaying pro-inflammatory properties. It is suggested that increased neutrophil could inhibit T cell activation and eliminate lymphocyte-mediated immune response by producing arginase, nitric oxide and reactive oxygen species [21]. Intriguingly, Wu et al. implied that the proportions of circulating naïve CD4+ T cells were higher in ACLF patients than in No-ACLF HBV patients, whereas the fractions of activated and differentiated T cells were preferentially depleted [22]. Specifically, the proportions of CD45RA-CCR-7+ (Central Memory) and CD45RA-CCR-7-(Effector Memory) CD4+ T cells were significantly lower in the circulation in patients with ACLF. The authors hypothesized that it can be attributed to recruitment and sequestration of differentiated CD4+ T cells within the hepatic compartment, and subsequently inducing liver inflammation.

Another study confirmed a strong correlation between high NLR and elevated cytokines in patients with colorectal cancer [16]. It is conceivable that our findings bolster this cumulative evidence. We revealed that two cytokines, referring to IL-6 and IL-8, were significantly upregulated in cirrhotics in comparison to healthy controls. When determined in the circulation, these two cytokines appeared positive correlation with NLR. Taken together, we substantiate that NLR-associated cytokine profile may partly portend immune dysregulation related to severity at both cellular and non-cellular levels in patients with LC.

A growing body of literature have implicated the impact of circulating cytokines in evaluating the cirrhosis severity and prognosis. However, the results were heterogeneous owing to variable recruited population and distinct measured methods. Although a tendency towards increasing levels of IL-6 and IL-8 with aggravating CTP was detected, the difference did not reach statistical significance in a study conducted by Wiese et al. [23] In agreement with our results, Dirchwolf et al. showed a sustained response of IL-6 and IL-8 in decompensated cirrhosis who accordingly developed ACLF [24]. IL-6 is the most explored serum marker of inflammation and consistently proved to be associated with prognosis, whilst IL-8 is known as permanent chemokine for neutrophils. These hallmarks may partly state the evidence in the present study, that is, majority of prognostic information resulted from elevated neutrophil count. Intriguingly, inflammatory cytokines released by neutrophils may result in lymphocyte apoptosis. On the other hand, relative lymphopenia implies physiologic stress, which can also enhance the production of pro-inflammatory cytokines [25]. Another issue that should be addressed is we did not observe significant change of IL-10 between healthy and cirrhotic subjects, in contrast to previous findings [24]. Although interpretation for these contradictory results was unclear, the relatively small proportion of patients at advanced stage with available serum samples should be taken into account.

Our nomogram also included albumin and MELD, which comprehensively reflecting laboratory indices. As LC advanced, the decline of albumin was associated with deterioration of hepatic function reserve as well as systemic inflammatory response [26]. Some investigators showed that patients with hypoalbuminemia were susceptible to infection and prone to sepsis [27,28]. Myers et al. analyzed 40,393

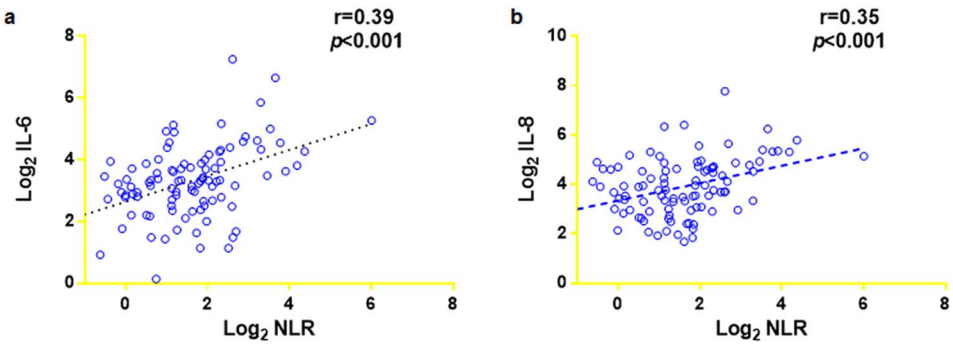

Fig. 5. Circulating IL-6 and IL-8 correlated (*nonparametric correlation*) moderately with the expression levels of NLR in cirrhotics from the validation cohort. Spearman's rank correlation coefficients [rho (r)] were indicated.

patients registered for liver transplantation in the U.S. from 2002 and 2007, and noted that modification of MELD to include serum albumin can improve predictive performance of 3-month mortality [29]. Additionally, a study by Zhang et al. also investigated the role of NLR for predicting early mortality in HBV-related decompensated cirrhotic patients [30]. They concluded that NLR and MELD were both independent predictors for 30-day mortality, which was highly consistent with our findings. A combination of these two indices augmented the predictive power. Furthermore, they observed that an increased frequency of liver related complications in higher NLR group. Collectively, although the physiological association between an elevated NLR and poor prognosis is complex and remains to be elucidated, the newly intensified hepatic inflammation during flare-ups of liver injury may be partly responsible. As a matter fact, the established nomogram demonstrated superior discrimination power (C-index 0.88) than incorporating albumin or MELD alone. Moreover, a slightly higher C-index (0.89) was seen while performing internal validation in another set of cirrhotics. Taken together, the rationale of selected variables had been partly confirmed.

Several unique features of the present study should be addressed as follows: (1) By reviewing the literature exploring prognostication of NLR in liver diseases, no consensus exists regarding cut-off values among a variety of study population and distinct outcomes. These conclusions were so heterogeneous that no availability and credibility could be warranted. As expected, liver cirrhosis is not a temporally-fixed entity, patients may progress or improve in a dynamic fashion. Therefore we integrated three continuous parameters, but not arbitrarily discrete demarcation, into the nomogram. We considered this model was a refined option for keeping LC patients under constant surveillance, as well as prediction of the 30-day mortality at admission. (2) We did not exclude LC patients precipitated by infection, which might contribute to the difference in the predictive values of baseline NLR between previous studies and ours. The nomogram proposed by us is more convenient and universal for practitioner, as it reflects the “real world” daily practice from a wide range of practice settings and minimize patient selection or potential referral bias. Indeed endotoxaemia and bacterial translocation are characteristic of decompensated cirrhosis independent of overt infection [31]. Moreover, NLR may serve as an indicator of subclinical endotoxaemia [32]. Our results and others do not contradict *per se*, with the initial NLR reflecting both the disease itself and the external environment factors in the current study [25]. (3) To the best of our knowledge, this is the first publication that orchestrates a moderate correlation of NLR with IL-6/IL-8. These data have identified static NLR as a grim prognostic indicator of immune dysregulation, which suggesting its potential for selecting acute deteriorated LC cases for experimental therapeutics in early phase clinical trials. (4) Nomogram is a simple pictorial representation of a complex mathematical formula [10]. It generates a probability of a clinical event and allows clinician to clearly communicate with a patient using individual nomogram estimate. In fact, a study by Kanwal et al. examined temporal trends in the overall outcomes for hospitalized cirrhosis patients in the U.S. [33]. They concluded liver disease severity-related factors were the key drivers of mortality, and their effects appeared strongest during and 30 days following hospitalization. For instance, patients with MELD score $\geq$ 20 had 13- and 7-fold higher risk of in-hospital and 30-day mortality compared with those with MELD $<$ 10. Collectively, current nomogram included NLR (partly reflecting CAID), albumin and MELD, and inclusion of these factors would assist in an informed decision and clinical judgment.

Our study still has several limitations. First, this study was designed retrospectively in nature. Second, there were only 25 deaths in the entire cohort, which rendering the study underpowered. Actually, this is an ongoing longitudinal research with active enrollment of cirrhotics, we preferentially investigate the dynamic change of NLR to provide a more accuracy prognosis in future work [34,35]. Third, from the 117 patients in the validation cohort, serum cytokines profile were only available in 98 cases. Fourth, whether restoring the deregulated immune balance may serve as a therapeutic approach for LC patients with acute insults is not known. However, the association of NLR with short-term mortality, would make such pharmacologic interventions, much like antibiotics or immunomodulation, particularly attractive. Finally, we did not observe differential expression of other cytokines except for IL-6/IL-8 between health controls and cirrhotics at advanced stages. Indeed the baseline expression level of circulating cytokines were highly heterogeneous in the current body of literature, probably due to lack of consistent inclusion criteria or measures employed by investigators. Taken together, we consider it of upmost importance to confirm and extend our nomogram prospectively in multi-center clinical trials.

In summary, this study concluded that NLR was an independent predictor for 30-day mortality in LC patients with acute decompensation. The expression of NLR was associated with IL-6 and IL-8, two mainstays of circulating cytokines. A refined nomogram with NLR, MELD and albumin may be beneficial for clinician and patients in daily clinical settings, in terms of individualized stratification and therapeutic strategies.

Funding

This work was supported by the National Natural Science Foundation of China (grant number 81500397 to C.S. and grant number 81600509 to R.L.)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2018.01.007.

References

[1] A. Albillos, M. Lario, M. Alvarez-Mon, Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance, J. Hepatol. 61 (2014) 1385–1396.
[2] V. Arvaniti, G. D'Amico, G. Fede, et al., Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis, Gastroenterology 139 (2010) 1246–1256 56 e1–5.
[3] R. Moreau, R. Jalan, P. Gines, et al., Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis, Gastroenterology 144 (2013) 1426–1437 37 e1–9.
[4] M. Dirchwolf, A.E. Ruf, Role of systemic inflammation in cirrhosis: from pathogenesis to prognosis, World J. Hepatol. 7 (2015) 1974–1981.
[5] P. Avanzas, J. Quiles, E. Lopez de Sa, et al., Neutrophil count and infarct size in patients with acute myocardial infarction, Int. J. Cardiol. 97 (2004) 155–156.
[6] T.C. Xue, L. Zhang, X.Y. Xie, et al., Prognostic significance of the neutrophil-to-lymphocyte ratio in primary liver cancer: a meta-analysis, PLoS One 9 (2014) e96072.
[7] Y.J. Cai, J.J. Dong, J.Z. Dong, et al., A nomogram for predicting prognostic value of inflammatory response biomarkers in decompensated cirrhotic patients without acute-on-chronic liver failure, Aliment. Pharmacol. Ther. 45 (2017) 1413–1426.
[8] N. Alkhouri, G. Morris-Stiff, C. Campbell, et al., Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease, Liver Int. 32 (2012) 297–302.
[9] M.T. Kuo, T.H. Hu, S.N. Lu, et al., Neutrophil-to-lymphocyte ratio as a predictor of response to peginterferon plus ribavirin therapy for chronic hepatitis C, Dis. Markers 2014 (462958) (2014).
[10] V.P. Balachandran, M. Gonen, J.J. Smith, et al., Nomograms in oncology: more than meets the eye, Lancet Oncol. 16 (2015) e173–80.
[11] A. Iasonos, D. Schrag, G.V. Raj, et al., How to build and interpret a nomogram for cancer prognosis, J. Clin. Oncol. 26 (2008) 1364–1370.
[12] R.C. Chen, X.D. Wang, J.Z. Dong, et al., A MELD-based nomogram for predicting 3-month mortality of patients with acute-on-chronic hepatitis B liver failure, Clin. Chim. Acta 468 (2017) 195–200.
[13] K.Q. Shi, Y.J. Cai, Z. Lin, et al., Development and validation of a prognostic nomogram for acute-on-chronic hepatitis B liver failure, J. Gastroenterol. Hepatol. 32 (2017) 497–505.
[14] T. Horvatits, A. Drolz, K. Roedl, et al., Serum bile acids as marker for acute decompensation and acute-on-chronic liver failure in patients with non-cholestatic cirrhosis, Liver Int. 37 (2017) 224–231.
[15] S.K. Sarin, C.K. Kedarisetty, Z. Abbas, et al., Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014, Hepatol. Int. 8 (2014) 453–471.
[16] Z.Y. Chen, K. Raghav, C.H. Lieu, et al., Cytokine profile and prognostic significance of high neutrophil-lymphocyte ratio in colorectal cancer, Br. J. Cancer 112 (2015)

1088–1097.
[17] D. Benedeto-Stojanov, A. Nagorni, G. Bjelakovic, et al., The model for the end-stage liver disease and Child-Pugh score in predicting prognosis in patients with liver cirrhosis and esophageal variceal bleeding, Vojnosanit. Pregl. 66 (2009) 724–728.
[18] P. Cassano, E. Bui, A.H. Rogers, et al., Inflammatory cytokines in major depressive disorder: a case-control study, Aust. N. Z. J. Psychiatry 51 (2017) 23–31.
[19] J.H. Kwon, J.W. Jang, Y.W. Kim, et al., The usefulness of C-reactive protein and neutrophil-to-lymphocyte ratio for predicting the outcome in hospitalized patients with liver cirrhosis, BMC Gastroenterol. 15 (146) (2015).
[20] A. Kalra, J.P. Wedd, K.M. Bambha, et al., Neutrophil-to-lymphocyte ratio correlates with proinflammatory neutrophils and predicts death in low model for end-stage liver disease patients with cirrhosis, Liver Transpl. 23 (2017) 155–165.
[21] I. Muller, M. Munder, P. Kropf, et al., Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? Trends Immunol. 30 (2009) 522–530.
[22] W. Wu, H. Yan, H. Zhao, et al., Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: differentiate it from no-ACLF, Liver Int. (2017), http://dx.doi.org/10.1111/liv.13504 (Epub ahead of print).
[23] S. Wiese, C. Mortensen, J.P. Gotze, et al., Cardiac and proinflammatory markers predict prognosis in cirrhosis, Liver Int. 34 (2014) e19–30.
[24] M. Dirchwolf, A. Podhorzer, M. Marino, et al., Immune dysfunction in cirrhosis: distinct cytokines phenotypes according to cirrhosis severity, Cytokine 77 (2016) 14–25.
[25] Z. Guo, S. Yu, L. Xiao, et al., Dynamic change of neutrophil to lymphocyte ratio and hemorrhagic transformation after thrombolysis in stroke, J. Neuroinflammation 13 (199) (2016).
[26] V. Arroyo, R. Garcia-Martinez, X. Salvatella, Human serum albumin, systemic inflammation, and cirrhosis, J. Hepatol. 61 (2014) 396–407.
[27] A. Morotti, S. Marini, U.K. Lena, et al., Significance of admission hypoalbuminemia in acute intracerebral hemorrhage, J. Neurol. 264 (2017) 905–911.
[28] M. Yin, L. Si, W. Qin, et al., Predictive value of serum albumin level for the prognosis of severe sepsis without exogenous human albumin administration, J. Intensive Care Med. (2016), http://dx.doi.org/10.1177/0885066616685300 (Epub ahead of print).
[29] R.P. Myers, A.A. Shaheen, P. Faris, et al., Revision of MELD to include serum albumin improves prediction of mortality on the liver transplant waiting list, PLoS One 8 (2013) e51926.
[30] H. Zhang, Q. Sun, W. Mao, et al., Neutrophil-to-lymphocyte ratio predicts early mortality in patients with HBV-related decompensated cirrhosis, Gastroenterol. Res. Pract. 2016 (2016) 4394650.
[31] H.W. Zimmermann, P.A. Reuken, A. Koch, et al., Soluble urokinase plasminogen activator receptor is compartmentally regulated in decompensated cirrhosis and indicates immune activation and short-term mortality, J. Intern. Med. 274 (2013) 86–100.
[32] J.A. Leithead, N. Rajoriya, B.K. Gunson, et al., Neutrophil-to-lymphocyte ratio predicts mortality in patients listed for liver transplantation, Liver Int. 35 (2015) 502–509.
[33] F. Kanwal, A. Tansel, J.R. Kramer, et al., Trends in 30-day and 1-year mortality among patients hospitalized with cirrhosis from 2004 to 2013, Am. J. Gastroenterol. 112 (2017) 1287–1297.
[34] W. Zhao, Z. Wu, Y. Li, et al., Pretreatment neutrophil-to-lymphocyte ratio and its dynamic changes are associated with the overall survival in advanced cancer patients undergoing palliative care, Sci. Rep. 6 (31394) (2016).
[35] K.M. Cho, H. Park, D.Y. Oh, et al., Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and their dynamic changes during chemotherapy is useful to predict a more accurate prognosis of advanced biliary tract cancer, Oncotarget 8 (2017) 2329–2341.