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

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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

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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,