Clin. Lab. 2026;72:XXX-XXX
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ORIGINAL ARTICLE

A Nomogram Based on Blood Cell Analysis for Differentiating Simple Fatty Liver from Fatty Liver Cirrhosis

Yazhou He [1, *], Xingkai Tang [2, *], Ting Shi [3], Zhilong Liu [2]

[*] *Yazhou He and Xingkai Tang contributed equally to this work as co-first authors*
[1] *Department of Laboratory Medicine, Yingshan County People's Hospital, Nanchong, China*
[2] *Department of Laboratory Medicine, Dazhou Integrated Traditional Chinese Medicine & Western Medicine Hospital, Dazhou Second People's Hospital, Dazhou, China*
[3] *Department of Critical Care Medicine, Yingshan County People's Hospital, Nanchong, China*

ABSTRACT

Background: **Fatty liver disease affects 25 - 30% of the global population and can progress to cirrhosis. Current cirrhosis risk assessment tools face significant limitations including invasiveness, high costs, and limited accessibility. This study aimed to develop and validate a nomogram based on routine blood cell analysis parameters to distinguish between simple fatty liver and fatty liver cirrhosis.**

Methods: **This retrospective study included 433 patients with fatty liver disease from two tertiary care hospitals between January and December 2024. Patients from Yingshan County People's Hospital (n = 212) comprised the training set, while those from Dazhou Integrated Traditional Chinese and Western Medicine Hospital (n = 221) formed the external validation set. Complete blood count parameters and derived inflammatory indices were collected. Univariable and multivariable logistic regression analyses identified independent risk factors of cirrhosis. A nomogram was constructed and validated using receiver operating characteristic curve analysis, calibration plots, and decision curve analysis.**

Results: **In the training set, 34 patients (16.0%) had cirrhosis. Multivariable analysis identified four independent risk factors: basophil percentage (OR = 4.417, 95% CI: 1.205 - 18.177, p = 0.029), red blood cell distribution width-coefficient of variation (OR = 1.552, 95% CI: 1.128 - 2.172, p = 0.007), neutrophil-to-lymphocyte ratio (OR = 2.121, 95% CI: 1.123 - 4.116, p = 0.022), and lymphocyte percentage (OR = 1.110, 95% CI: 0.999 - 1.231, p = 0.047). The nomogram demonstrated good differentiation in the training set (AUC = 0.763, 95% CI: 0.684 - 0.841) and acceptable performance in the external validation set (AUC = 0.706, 95% CI: 0.613 - 0.799). The corresponding negative predictive values were 93.9% in the training set and 86.3% in the validation set, although the positive predictive values were not ideal. Calibration plots showed excellent agreement between predicted and observed outcomes in both sets. Decision curve analysis confirmed a favorable net benefit across clinically relevant threshold probabilities.**

Conclusions: **This study developed and externally validated a simple, cost-effective nomogram incorporating routine blood cell analysis parameters to differentiate between simple fatty liver and fatty liver disease with concomitant cirrhosis. Although the model's positive predictive value was not ideal, its high negative predictive value effectively helps to rule out cirrhosis in patients with fatty liver disease. The model demonstrates good differentiation, calibration, and clinical utility, providing a practical tool for risk stratification that can be seamlessly integrated into routine clinical workflows, particularly in resource-limited settings.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.251139)

Correspondence:
Zhilong Liu
Department of Laboratory Medicine
Dazhou Integrated Traditional Chinese Medicine & Western Medicine Hospital
Dazhou
Sichuan Province, 635000
China
Phone/Fax: + 86 8182350072
Email: liuzhilong@gris.org.cn

Manuscript accepted December 9, 2025

KEYWORDS

fatty liver disease, cirrhosis, nomogram, blood cell analysis

INTRODUCTION

Fatty liver disease has emerged as one of the most prevalent chronic liver conditions worldwide, affecting approximately 25 - 30% of the global population, with its incidence continuing to rise in parallel with the obesity and metabolic syndrome epidemics [1]. Non-alcoholic fatty liver disease (NAFLD) and alcohol-related liver disease (ALD) represent the two major etiologies, both characterized by hepatic steatosis that can progress through a spectrum of pathological stages [2]. While many patients remain in the relatively benign stage of simple steatosis, a substantial proportion progresses to steatohepatitis, advanced fibrosis, and ultimately cirrhosis, which represents the most critical adverse outcome in the natural history of fatty liver disease [3,4]. Cirrhosis development fundamentally alters patient prognosis, leading to hepatic decompensation, portal hypertension, hepatocellular carcinoma, and significantly increased mortality, with 5-year survival rates dropping to approximately 50% once decompensation occurs [5,6]. Early and accurate differentiation between simple fatty liver and fatty liver with concomitant cirrhosis holds positive clinical significance for precise diagnosis and the formulation of subsequent treatment strategies [7]. Despite the clinical importance of differentiating cirrhosis risk in fatty liver disease patients, current assessment tools face significant limitations in routine clinical practice. Liver biopsy, considered the gold standard for fibrosis staging, is invasive, expensive, subject to sampling error, and carries procedural risks, making it unsuitable for widespread screening or serial monitoring [8]. Non-invasive alternatives such as transient elastography and magnetic resonance elastography, while valuable, require specialized equipment, technical expertise, and substantial costs that limit their accessibility, particularly in resource-limited settings and primary care environments [9]. Serum-based fibrosis scores, including the FIB-4 index and NAFLD fibrosis score, have been developed and validated; however, these models often require multiple biochemical parameters and may have suboptimal performance in specific populations or disease stages [10]. Furthermore, existing models frequently overlook readily available hematological parameters that reflect systemic inflammation, immune dysregulation, and bone marrow dysfunction-all of which are intimately linked to liver fibrosis progression [7,9]. There remains an unmet need for simple, cost-effective, and widely accessible tools that can be seamlessly integrated into routine clinical workflows to accurately identify patients with fatty liver disease who already have concomitant cirrhosis.

Complete blood count (CBC) analysis represents one of the most fundamental and universally available laboratory tests, routinely performed in clinical practice with minimal cost and rapid turnaround time [10]. Emerging evidence suggests that CBC-derived parameters and inflammatory indices may serve as valuable biomarkers across various disease states. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) have demonstrated prognostic value in cardiovascular diseases, malignancies, and inflammatory conditions, reflecting the balance between systemic inflammation and immune response [11,12]. In liver disease specifically, these indices have been associated with disease severity and outcomes in viral hepatitis and hepatocellular carcinoma [8]. Additionally, red blood cell distribution width (RDW), traditionally used to classify anemia, has emerged as a marker of oxidative stress and inflammation, showing associations with liver fibrosis severity [13]. Alterations in lymphocyte and monocyte populations reflect immune dysfunction in chronic liver disease, while basophil changes may indicate bone marrow response to chronic inflammation. Despite these observations, comprehensive diagnosis models integrating multiple CBC parameters specifically for cirrhosis risk stratification in fatty liver disease patients remain limited. Therefore, this study aimed to develop and externally validate a nomogram incorporating routine blood cell analysis parameters to identify patients with fatty liver disease who have concomitant cirrhosis, providing a practical and accessible tool for clinical decision-making and risk stratification.

MATERIALS AND METHODS

This retrospective study was conducted at two tertiary care hospitals between January and December 2024. Patients recruited from Yingshan County People's Hospital comprised the training set, whereas those from Dazhou Integrated Traditional Chinese and Western Medicine Hospital formed the external validation set. To ensure data comparability across centers, identical data collection protocols and standardized operating procedures were implemented at both institutions. Hematological parameters were measured using the same automated hematology analyzer (Mindray BC-7500CS, Shenzhen, China) at both sites. All instruments were calibrated according to the manufacturer's specifications and were maintained under rigorous quality control programs throughout the study period. The study protocol received approval from the Institutional Review Boards of both participating institutions. Given the retrospective nature of this investigation, the requirement for informed consent was waived in accordance with institutional guidelines.

Patient selection

Eligible patients were aged ≥ 18 years, with fatty liver disease confirmed by imaging or histopathology. All enrolled patients were stratified into cirrhosis and non-cirrhosis groups. Cirrhosis status was determined by liver biopsy, transient elastography, or comprehensive clinical assessment. Patients were required to have complete blood cell analysis parameters obtained within 1 month of diagnosis and liver function tests within 3 months of diagnosis. Exclusion criteria comprised: 1) viral hepatitis (hepatitis B or C virus infection); 2) autoimmune or hereditary metabolic liver diseases; 3) drug-induced liver injury; 4) hepatic or extrahepatic malignancy; 5) hematological disorders, including hematologic malignancies, severe anemia, or non-cirrhotic thrombocytopenia/thrombocytosis; 6) acute infection or major surgery within 3 months prior to enrollment; 7) acute decompensation of cirrhosis; 8) severe cardiac dysfunction, renal insufficiency, or respiratory failure; 9) poorly controlled diabetes mellitus; 10) use of medications known to affect blood cell counts within 3 months; 11) hemolyzed or coagulated blood samples; 12) incomplete clinical or laboratory data; and 13) pregnancy or lactation.

Data collection

Clinical data were extracted from electronic medical records by trained researchers at each center using standardized case report forms. The following variables were collected: 1) demographic characteristics, including age and gender; 2) complete blood count parameters, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW); 3) differential white blood cell counts, including absolute counts and percentages of neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), and basophils (BASO); and 4) derived inflammatory indices, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII).

Quality control

Both participating laboratories implemented comprehensive internal and external quality control measures. For internal quality control, third-party quality control materials were used for blood counts, with monitoring performed according to Levey-Jennings rules. All samples were analyzed only after internal quality control results fell within acceptable ranges.

Externally, both laboratories have participated continuously for over five years in external quality assessment (EQA) programs administered by the Health Commission of Sichuan Province and the National Center for Clinical Laboratories (NCCL), including specific schemes for differential blood counts. Satisfactory performance has been consistently maintained in all EQA evaluations.

Statistical analysis

All statistical analyses were conducted using R version 4.5.0. Categorical variables were expressed as frequencies with percentages and compared between groups using the chi-squared test or Fisher's exact test as appropriate. Continuous variables were assessed for normality using the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation (SD) and compared using independent samples *t*-tests or Welch's *t*-tests depending on the homogeneity of variances assessed by Levene's test. Non-normally distributed variables were expressed as median with interquartile range (IQR) and compared using the Mann-Whitney U test. Univariable logistic regression analyses were conducted in the training set to identify potential risk factors of cirrhosis. Variables with $p < 0.05$ in univariable analysis were considered as candidates for the multivariable model. Multicollinearity among candidate variables was assessed using variance inflation factors (VIF), with VIF > 10 indicating significant multicollinearity. When highly correlated variables were identified, only the variable demonstrating the strongest association with the outcome was retained for further analysis. Multivariable logistic regression with backward stepwise selection (retention criterion: $p < 0.05$) was then performed to identify independent risk factors of cirrhosis. A nomogram was constructed based on the final multivariable logistic regression model to provide a visual tool for cirrhosis diagnosis. Model performance was evaluated in both the training and external validation sets. Differentiation was assessed using receiver operating characteristic (ROC) curve analysis with calculation of the area under the curve (AUC). Calibration was evaluated using calibration plots to assess the agreement between predicted probabilities and observed outcomes. Clinical utility was determined using decision curve analysis (DCA) to quantify the net benefit across different threshold probabilities. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Comparison of clinical data between the training and validation sets

A total of 433 patients with fatty liver disease from two centers were included in this study. Data from one center were used as the training set (n = 212), while data from the other center served as the validation set (n = 221). The comparison of demographic characteristics and blood cell analysis parameters between the two groups is shown in Table 1. The two groups showed comparable distributions in cirrhosis prevalence ($p = 0.248$), gender ($p = 0.122$), and age ($p = 0.168$). Most blood cell analysis parameters demonstrated good com-

Table 1. Comparison of demographic characteristics and blood cell analysis parameters between training and validation sets.

Parameters	Training set (n = 212)	Validation set (n = 221)	p-value
Cirrhosis, n (%)			
Yes	34 (16.0%)	46 (20.8%)	0.248
No	178 (84.0%)	175 (79.2%)	
Gender, n (%)			
Female	126 (59.4%)	114 (51.6%)	0.122
Male	86 (40.6%)	107 (48.4%)	
Age (years), mean (SD)	57.33 (13.88)	55.45 (14.43)	0.168
WBC (10^9/L), mean (SD)	7.21 (2.15)	7.41 (2.42)	0.362
LYM (%), mean (SD)	29.81 (9.28)	31.34 (8.57)	0.075
MOMO (%), mean (SD)	7.87 (2.06)	8.12 (2.19)	0.216
NEU (%), mean (SD)	58.71 (9.81)	57.00 (9.05)	0.061
EOS (%), mean (SD)	2.93 (1.47)	2.87 (1.82)	0.761
BASO (%), mean (SD)	0.81 (0.33)	0.80 (0.31)	0.726
LYM count (× 10^9/L), mean (SD)	2.13 (0.87)	2.27 (0.81)	0.094
MONO count (× 10^9/L), mean (SD)	0.55 (0.19)	0.59 (0.21)	0.073
NEU count (× 10^9/L), mean (SD)	4.26 (1.55)	4.29 (1.78)	0.836
EOS count (× 10^9/L), mean (SD)	0.21 (0.05)	0.21 (0.05)	0.873
BASO count (× 10^9/L), mean (SD)	0.05 (0.01)	0.06 (0.02)	0.396
RBC (× 10^{12}/L), mean (SD)	4.68 (0.50)	4.74 (0.54)	0.216
HB (g/L), mean (SD)	13.97 (1.64)	14.09 (1.69)	0.434
HCT (%), mean (SD)	41.30 (4.51)	41.86 (4.63)	0.204
MCV (fL), mean (SD)	88.46 (5.73)	88.64 (6.60)	0.758
MCHC (g/L), mean (SD)	33.78 (0.94)	33.62 (0.92)	0.090
MCH (pg), mean (SD)	29.89 (2.33)	29.83 (2.66)	0.802
RDW-CV (%), mean (SD)	14.07 (1.45)	14.03 (1.37)	0.722
PLT (× 10^9/L), mean (SD)	245.10 (73.25)	239.45 (74.55)	0.427
MPV (fL), mean (SD)	8.20 (0.99)	8.32 (0.90)	0.194
NRBC (/100 WBC), mean (SD)	0.08 (0.02)	0.09 (0.03)	0.332
hsCRP (mg/L), mean (SD)	4.43 (6.15)	5.02 (7.66)	0.378
NLR, mean (SD)	2.33 (1.34)	2.06 (1.00)	0.018 *
PLR, mean (SD)	8.99 (3.80)	8.21 (3.64)	0.029 *
SII, mean (SD)	551.92 (307.28)	487.64 (280.69)	0.023 *

WBC: white blood cell count, LYM: lymphocyte, MONO: monocyte, NEU: neutrophil, EOS: eosinophil, BASO: basophil, RBC: red blood cell count, HB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, RDW-CV: red blood cell distribution width-coefficient of variation, PLT: platelet count, MPV: mean platelet volume, NRBC: nucleated red blood cell, hsCRP: high-sensitivity C-reactive protein, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammation index. * Statistically significant at $p < 0.05$.

parability, including WBC count and differentials, red blood cell parameters, platelet-related indices, NRBC, and hsCRP levels (all $p > 0.05$). Three derived inflammatory indices showed statistically significant differences: NLR ($p = 0.018$), PLR ($p = 0.029$), and SII ($p = 0.023$). However, their constituent parameters showed no significant differences, suggesting minimal impact on model generalizability.

Comparison of clinical data between cirrhosis and non-cirrhosis groups in the training set

To identify potential biomarkers associated with cirrhosis, patients in the training set were divided into cirrhosis (n = 34) and non-cirrhosis (n = 178) groups. The comparison is presented in Table 2. No significant differences were observed in gender distribution ($p = 0.787$) or age ($p = 0.976$) between the two groups. The

Table 2. Comparison of hematological parameters between cirrhosis and non-cirrhosis groups in the training set.

Parameters	Non-cirrhosis group (n = 178)	Cirrhosis group (n = 34)	p-value
Gender, n (%)			
Female	107 (60.1%)	19 (55.9%)	0.787
Male	71 (39.9%)	15 (44.1%)	
Age (years), mean (SD)	57.34 (14.52)	57.26 (10.02)	0.976
WBC (10^9/L), mean (SD)	7.32 (2.18)	6.60 (1.88)	0.071
LYM (%), mean (SD)	30.37 (8.67)	26.87 (11.73)	0.044 *
MONO (%), mean (SD)	7.71 (1.77)	8.71 (3.07)	0.009 *
NEU (%), mean (SD)	58.43 (9.27)	60.18 (12.31)	0.342
EOS (%), mean (SD)	2.83 (2.20)	3.46 (3.56)	0.167
BASO (%), mean (SD)	0.78 (0.34)	0.92 (0.25)	0.030 *
LYM count (× 10^9/L), mean (SD)	2.20 (0.85)	1.75 (0.92)	0.005 *
MONO count (× 10^9/L), mean (SD)	0.55 (0.19)	0.56 (0.24)	0.869
NEU count (× 10^9/L), mean (SD)	4.31 (1.57)	4.02 (1.49)	0.329
EOS count (× 10^9/L), mean (SD)	0.21 (0.05)	0.22 (0.04)	0.769
BASO count (× 10^9/L), mean (SD)	0.05 (0.05)	0.06 (0.05)	0.464
RBC (× 10^{12}/L), mean (SD)	4.73 (0.47)	4.42 (0.54)	0.001 *
HB (g/L), mean (SD)	14.11 (1.47)	13.21 (2.19)	0.003 *
HCT (%), mean (SD)	41.73 (4.10)	39.04 (5.82)	0.001 *
MCV (fL), mean (SD)	88.44 (4.75)	88.54 (9.40)	0.929
MCHC (g/L), mean (SD)	33.79 (0.89)	33.72 (1.20)	0.689
MCH (pg), mean (SD)	29.89 (2.00)	29.90 (3.63)	0.987
RDW-CV (%), mean (SD)	13.93 (1.27)	14.85 (2.00)	0.001 *
PLT (× 10^9/L), mean (SD)	250.81 (68.23)	215.21 (90.79)	0.009 *
MPV (fL), mean (SD)	8.17 (0.95)	8.41 (1.16)	0.196
NRBC (/100 WBC), mean (SD)	0.08 (0.02)	0.09 (0.01)	0.393
hsCRP (mg/L), mean (SD)	4.29 (2.90)	5.16 (2.39)	0.451
NLR, mean (SD)	2.20 (1.07)	3.04 (2.15)	0.001 *
PLR, mean (SD)	8.94 (3.64)	9.25 (4.59)	0.665
SII, mean (SD)	545.04 (295.81)	587.94 (364.38)	0.457

WBC: white blood cell count, LYM: lymphocyte, MONO: monocyte, NEU: neutrophil, EOS: eosinophil, BASO: basophil, RBC: red blood cell count, HB: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, RDW-CV: red blood cell distribution width-coefficient of variation, PLT: platelet count, MPV: mean platelet volume, NRBC: nucleated red blood cell, hsCRP: high-sensitivity C-reactive protein, NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammation index. * Statistically significant at $p < 0.05$.

cirrhosis group showed significantly lower lymphocyte percentage ($p = 0.044$) and absolute count ($p = 0.005$) but higher monocyte percentage ($p = 0.009$) and basophil percentage ($p = 0.030$). Red blood cell parameters were significantly decreased in the cirrhosis group, including RBC count ($p = 0.001$), hemoglobin ($p = 0.003$), and hematocrit ($p = 0.001$), while RDW-CV was elevated ($p = 0.001$). Platelet count was significantly lower in the cirrhosis group ($p = 0.009$). Among derived inflammatory indices, only NLR was significantly higher in the cirrhosis group ($p = 0.001$). Other parameters showed no significant differences between the two groups (all $p > 0.05$). These findings indicate that cirrhosis in fatty liver disease is associated with alterations in immune cell distribution, anemia, increased red blood cell size variability, thrombocytopenia, and elevated systemic inflammation, which are consistent with the pathophysiological changes in advanced liver disease.

Table 3. Results of multivariate logistic regression for training set.

Variables	β	Standard error	p-value	OR	95% CI
Intercept	-8.635	4.278	0.043 *	< 0.001	/
LYM	0.104	0.053	0.047 *	1.110	0.999 - 1.231
MONO	0.111	0.100	0.265	1.118	0.921 - 1.369
BASO	1.485	0.681	0.029 *	4.417	1.205 - 18.177
LYM count	-0.243	0.402	0.545	0.784	0.337 - 1.644
RBC	-1.553	0.886	0.079	0.212	0.035 - 1.172
HB	1.028	0.717	0.151	2.797	0.698 - 11.834
HCT	-0.299	0.276	0.278	0.741	0.427 - 1.269
RDW-CV	0.439	0.163	0.007 *	1.552	1.128 - 2.172
PLT	-0.003	0.003	0.378	0.997	0.990 - 1.003
NLR	0.752	0.327	0.022 *	2.121	1.123 - 4.116

Statistical analysis was performed using multivariate logistic regression. LYM: lymphocyte, MONO: monocyte, BASO: basophil, RBC: red blood cell count, HB: hemoglobin, HCT: hematocrit, RDW-CV: red blood cell distribution width-coefficient of variation, PLT: platelet count, NLR: neutrophil-to-lymphocyte ratio. * Statistically significant at p < 0.05.

Table 4. ROC curve analysis results.

	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Training set	0.142	76.5	69.7	32.5	93.9
Validation set	0.184	45.7	89.7	53.8	86.3

PPV: positive predictive value, NPV: negative predictive value.

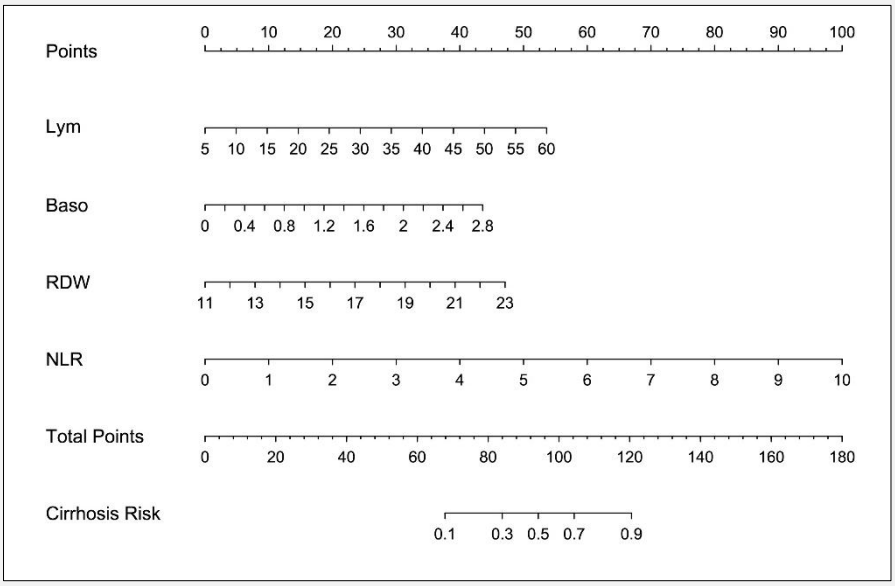


Figure 1. Nomogram model for diagnosing cirrhosis in patients with fatty liver disease based on the training set.

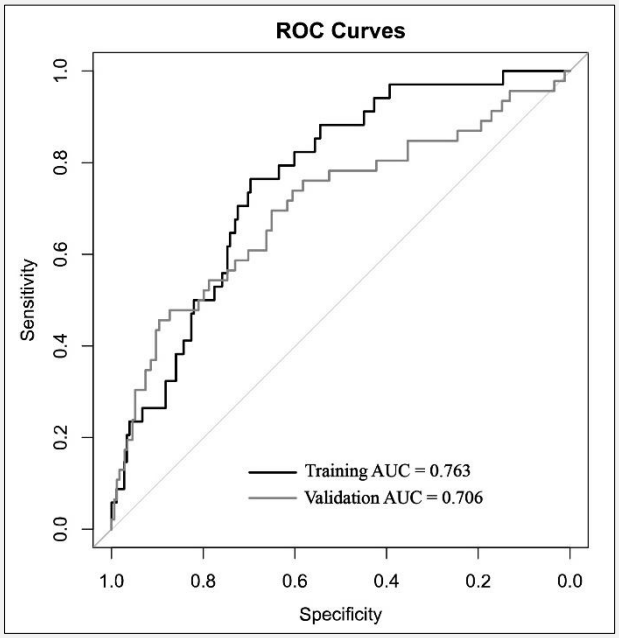


Figure 2. ROC curve analysis of the training and validation sets.

AUC: area under the curve, ROC: receiver operating characteristic.

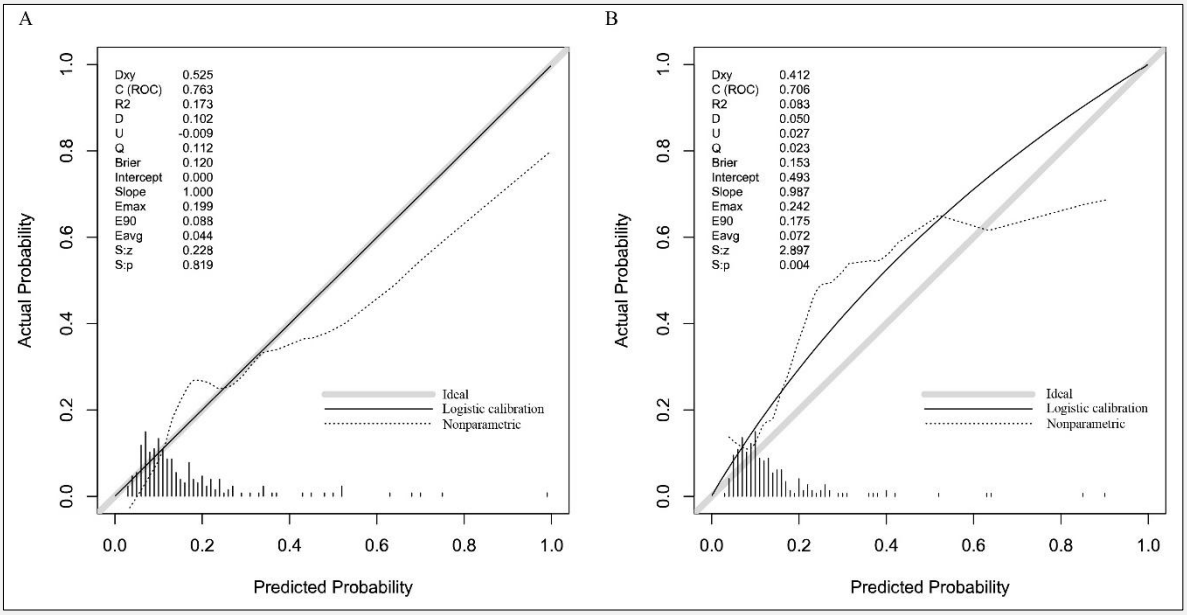


Figure 3. Calibration curve analysis of the training and validation sets.

A: Training set, B: validation set. The ideal line indicates perfect calibration. Logistic calibration and nonparametric calibration curves demonstrate the agreement between predicted and observed probabilities.

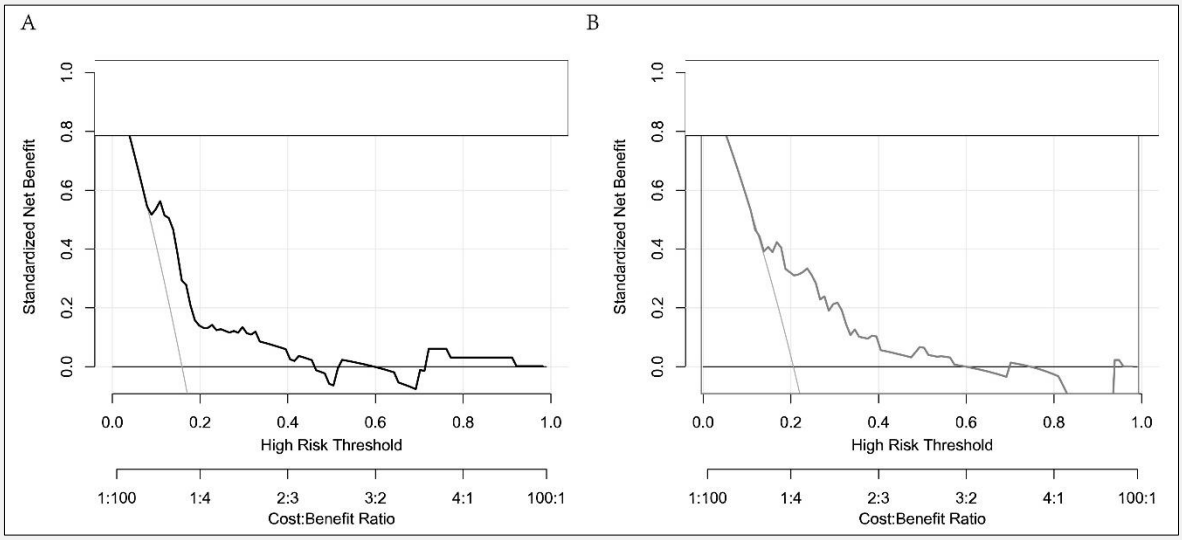


Figure 4. Decision curve analysis of the training and validation sets.

A: Training set, B: Validation set. The nomogram model shows superior net benefit compared with the treat-all and treat-none strategies across a range of threshold probabilities. The decision curve shows the net benefit of using the nomogram to guide further diagnostic evaluation compared to strategies of evaluating all patients ('treat all') or no patients ('treat none') across different threshold probabilities.

Multivariate logistic regression analysis for independent risk factors of cirrhosis

To identify independent risk factors of cirrhosis in fatty liver disease, variables with $p < 0.05$ in univariable analysis were entered into a multivariable logistic regression model with backward stepwise selection. The final model results are presented in Table 3. Four variables emerged as independent risk factors of cirrhosis: lymphocyte percentage (Lym%, OR = 1.110, 95% CI: 0.999 - 1.231, p = 0.047), basophil percentage (Baso%, OR = 4.417, 95% CI: 1.205 - 18.177, p = 0.029), red blood cell distribution width-coefficient of variation (RDW-CV, OR = 1.552, 95% CI: 1.128 - 2.172, p = 0.007), and neutrophil-to-lymphocyte ratio (NLR, OR = 2.121, 95% CI: 1.123 - 4.116, p = 0.022). Among these risk factors, elevated basophil percentage showed the strongest association with cirrhosis risk, with each unit increase associated with a 4.4-fold increase in cirrhosis odds. Similarly, higher RDW-CV and NLR were associated with increased cirrhosis risk, with ORs of 1.552 and 2.121, respectively. Lymphocyte percentage also retained statistical significance in the final model (p = 0.047). Other variables including monocyte percentage, lymphocyte count, RBC count, hemoglobin, hematocrit, and platelet count did not retain statistical significance in the multivariable model (all $p > 0.05$), suggesting their associations observed in univariable analysis were confounded by other factors.

Nomogram model construction

Based on the four independent risk factors identified in the multivariable logistic regression analysis, a nomogram diagnosis model was constructed to estimate individual cirrhosis risk in patients with fatty liver disease (Figure 1). The nomogram integrates lymphocyte percentage, basophil percentage, RDW-CV, and NLR. Each risk factor is represented by a point scale, where specific values correspond to point scores. Total points are calculated by summing up individual points from all four risk factors, which are then converted to cirrhosis probability using the bottom scale. To facilitate the clinical application of the nomogram, we have developed a static web-based risk calculator for clinicians, which is available for download in the Supplementary Material (https://doi.org/10.5281/zenodo.17837517).

Basophil percentage contributes the greatest weight to the model, consistent with its highest odds ratio, followed by NLR, RDW-CV, and lymphocyte percentage.

Model performance and validation

The differentiation ability of the nomogram was evaluated using receiver operating characteristic (ROC) curve analysis in both the training and validation sets (Figure 2). In the training set, the model demonstrated good differentiation performance with an area under the curve (AUC) of 0.763 (95% CI: 0.684 - 0.841), while in the external validation set, it maintained satisfactory differentiation ability with an AUC of 0.706 (95% CI: 0.613 -

0.799), indicating acceptable generalizability across different clinical settings. At the optimal cutoff value, the model showed limited positive predictive value in both cohorts; however, it achieved high negative predictive values of 93.9% in the training set and 86.3% in the validation set, indicating excellent performance in ruling out concomitant cirrhosis in patients with fatty liver disease (Table 4). Calibration performance was assessed using calibration plots in both sets (Figure 3). In the training set (Figure 3A), the calibration curve demonstrated excellent agreement between predicted probabilities and observed outcomes, with both the logistic and nonparametric calibration curves closely approximating the ideal 45-degree diagonal line. In the external validation set (Figure 3B), the model maintained acceptable calibration performance with reasonable concordance to the ideal line, though with slightly wider deviation compared to the training set. Decision curve analysis (DCA) was performed to evaluate the clinical utility of the diagnosis model (Figure 4). In the training set (Figure 4A), the nomogram demonstrated superior net benefit compared to the "treat all" and "treat none" strategies across threshold probabilities ranging from 0.1 to 0.6, while in the validation set (Figure 4B), similar patterns were observed with optimal net benefit at threshold probabilities between 0.1 and 0.5. Overall, these findings suggest that the nomogram provides reliable probability estimates and demonstrates substantial net benefit across a clinically relevant range of threshold probabilities in both cohorts. This supports its potential utility for clinical decision-making, particularly in excluding cirrhosis among patients with fatty liver disease.

DISCUSSION

This study developed and externally validated a novel nomogram incorporating four routine blood cell analysis parameters-basophil percentage, RDW-CV, NLR, and lymphocyte percentage-to identify concomitant cirrhosis in patients with fatty liver disease. The model demonstrated good differentiation (AUC = 0.763 in training set, 0.706 in validation set). Although the model showed limited positive predictive value, its excellent negative predictive value is of significant clinical importance for ruling out cirrhosis. These findings suggest that readily available hematological parameters can effectively distinguish patients with cirrhosis among those with fatty liver disease, offering a practical alternative to expensive or invasive assessment methods.

The identification of basophil percentage as the strongest differentiating variable (OR = 4.417) represents a novel finding in the identification of cirrhosis. While basophils constitute only 0.5 - 1% of circulating leukocytes, their elevation in cirrhosis patients may reflect complex immunological alterations in advanced liver disease [14]. Basophils release histamine and heparin, participate in allergic responses, and modulate immune reactions through cytokine production [15]. In chronic liver disease, basophilia may result from bone marrow stimulation by inflammatory mediators, altered hepatic clearance of basophil-stimulating factors, or compensatory responses to portal hypertension-induced splenic sequestration [16]. Although previous studies have documented basophil count alterations in various liver diseases, their specific role in distinguishing cirrhosis has been underexplored. Our findings suggest that basophil percentage warrants further investigation as a potential biomarker for hepatic fibrosis progression.

RDW-CV emerged as another differentiating variable (OR = 1.552), consistent with accumulating evidence linking elevated RDW to liver disease severity. RDW reflects heterogeneity in erythrocyte size and has been associated with oxidative stress, chronic inflammation, and impaired erythropoiesis-all hallmarks of cirrhosis [17]. In cirrhotic patients, multiple mechanisms contribute to elevated RDW, including nutritional deficiencies, hypersplenism-induced hemolysis, bone marrow suppression by inflammatory cytokines, and direct toxic effects of accumulated bile acids on erythrocyte membranes [18]. Several studies have demonstrated associations between RDW and liver fibrosis stages in NAFLD and chronic hepatitis C [19,20]. Our results extend these observations by incorporating RDW-CV into a comprehensive diagnostic model, reinforcing its utility as a simple, cost-effective marker for identifying cirrhosis.

The inclusion of NLR (OR = 2.121) aligns with extensive literature documenting its diagnostic and prognostic value across liver diseases. NLR reflects the balance between neutrophil-mediated inflammation and lymphocyte-mediated adaptive immunity, both of which are dysregulated in cirrhosis [21]. Elevated NLR in cirrhotic patients results from neutrophilia driven by systemic inflammation and bacterial translocation, coupled with lymphopenia secondary to splenic sequestration, apoptosis, and immunosenescence [10]. Previous studies have established NLR as a risk factors of mortality in cirrhosis and hepatocellular carcinoma development [22,23]. In NAFLD specifically, elevated NLR has been associated with advanced fibrosis and steatohepatitis [24]. Our findings corroborate these observations and demonstrate NLR's independent value in identifying cirrhosis when combined with other hematological parameters in a multivariable model.

Lymphocyte percentage showed a borderline significant association with cirrhosis risk (OR = 1.110, p = 0.047). While the direction of this association may appear counterintuitive, it likely reflects complex immunological dynamics in cirrhosis. Lymphopenia is well-documented in advanced liver disease due to splenic sequestration, increased apoptosis, and bone marrow dysfunction [25]. However, the relative lymphocyte percentage may paradoxically increase when absolute counts of other leukocyte populations decline more substantially, or when compensatory lymphocyte redistribution occurs in response to chronic antigenic stimulation from gut-derived bacterial products [26]. The modest effect size and borderline significance suggest that lymphocyte percen-

tage contributes limited independent information beyond other model components.

The nomogram's performance characteristics support its clinical applicability. The AUC of 0.763 in the training set and 0.706 in the external validation set indicate acceptable to good differentiation, comparable to established non-invasive fibrosis scores such as FIB-4 and NAFLD fibrosis score [27,28]. While slightly lower than some imaging-based modalities like transient elastography, our model offers distinct advantages in accessibility, cost-effectiveness, and ease of implementation [29]. The excellent calibration observed in both datasets confirms that predicted probabilities accurately reflect observed cirrhosis rates, essential for informed clinical decision-making. Decision curve analysis demonstrated net benefit across clinically relevant threshold probabilities (0.1 - 0.5), indicating that the model can improve patient outcomes when used to guide surveillance intensity, specialist referral, or therapeutic interventions. Several strengths characterize this study, including external validation in an independent cohort, exclusive utilization of routine CBC parameters universally available in clinical practice, an intuitive nomogram format easily interpretable by clinicians, and rigorous statistical methodology comprehensively evaluating model performance.

Several limitations warrant consideration. The retrospective design and relatively small sample size, particularly the limited number of cirrhosis cases, may affect model stability. The study population derived from two Chinese tertiary care centers may limit generalizability to other populations. To enhance the model's accessibility and applicability in primary care settings, we deliberately excluded biochemical parameters, relying solely on routine blood tests. While this design choice increases practicality, it may limit the model's diagnostic accuracy compared to approaches incorporating more comprehensive laboratory data. Notably, while the model demonstrated excellent performance in ruling out cirrhosis (high negative predictive value), its ability to confirm the diagnosis (positive predictive value) was limited. This performance profile suggests that its primary clinical utility may lie in exclusion rather than confirmation, which constitutes a significant limitation for broader application. Future research should address these limitations through prospective validation in diverse populations, incorporation of additional variables, and longitudinal studies evaluating cirrhosis progression.

CONCLUSION

This study developed and externally validated a simple, cost-effective nomogram incorporating four routine blood cell analysis parameters-basophil percentage, RDW-CV, NLR, and lymphocyte percentage-with the primary aim of accurately ruling out cirrhosis in patients with fatty liver disease. The model demonstrated good differentiation and excellent calibration in both training and validation cohorts. Its high negative predictive value underscores its clinical utility for exclusionary diagnosis. By leveraging universally available hematological parameters, this tool provides a practical and accessible option for initial screening, particularly in settings with limited resources, aiding in the early identification of low-risk patients and optimizing clinical decision-making. Further studies are warranted to confirm its generalizability.

Declaration of Generative AI in Scientific Writing:
No generative AI or AI-assisted technologies were used in the creation of this manuscript.

Supplementary Material:
The risk calculator based on the nomogram developed in this study has been archived on the Zenodo platform and assigned a permanent digital object identifier (https://doi.org/10.5281/zenodo.17837517). It is publicly accessible for validation and citation.

Declaration of Interest:
None of the authors have any commercial or other association that might pose a conflict of interest.

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