A Logistic Regression Model for The Prediction of HBV-Related Cirrhosis

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Research

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Abstract

**Background:** Cirrhosis is one of the most severe complications at the late stage of chronic HBV infection. The liver biopsy is the gold standard for the diagnosis of liver cirrhosis. However, a liver biopsy is associated with the risk of severe complications and a high cost. It is therefore necessary to find several biomarkers for the diagnosis of HBV-related cirrhosis.

**Methods:** The research was proceeded to evaluate the diagnostic value of hematological parameters to find the surrogate markers in HBV-related cirrhosis. Four validation sets were recruited from four centers (Zhongnan Hospital of Wuhan University; Tongji Hospital, Tongji Medical College of Huazhong University Science and Technology; Union Hospital, Tongji Medical College of Huazhong Science and Technology University; Renmin Hospital of Wuhan University) and were used to assess the performance of the model. They included 261 HBV-related cirrhosis patients and 288 healthy people. The four validation sets were generated to validate the logistic regression model and all showed good AUC with moderate specificities and sensitivities.

**Results:** The result show that the level of RDW, MPV, MPV/PC ratio, PLR and NLR were all significantly higher in HBV-related cirrhosis patients compared to healthy individuals. Most of these parameters owned a moderate AUC in HBV-related cirrhosis patients. However, their diagnostic sensitivities or specificities were unsatisfactory. Therefore, a logistic regression model was built by combining these hematological parameters. The model showed great diagnostic value with the AUC of 0.987, sensitivity of 96.1% and specificity of 95.1%. Besides, the other four validation sets were generated to validate the logistic regression model and all showed good AUC with moderate specificities and sensitivities.

**Conclusions:** The data indicate that the model might be substantially useful for the diagnosis of HBV-related cirrhosis.

Introduction

Cirrhosis is one of the most severe complications of chronic HBV infection especially at the late stage. The 1-year mortality rate of patients with cirrhosis varies from 1% (early-stage cirrhosis) to 57% (decompensated cirrhosis)

\[1-3]\). In addition, the 5-year cumulative hepatocellular carcinoma risk is 10%-17% in cirrhotic patients

\[4]\). Therefore, early diagnosis of HBV-related cirrhosis is crucial for early therapy and good prognosis

\[5-7]\).

The diagnosis of liver cirrhosis is traditionally based on the patients' manifestations, laboratory assays and imaging tests. However, these diagnostic methods have their limitations. Although the liver biopsy is the gold standard for cirrhosis diagnosis, it is invasive, difficult, expensive, and sometimes accompanied by significant morbidity

\[8,9]\). It is therefore not feasible for all patients. It is necessary to find biomarkers which can distinguish HBV-related cirrhosis in a non-invasive, accurate, and simple manner. Former studies have suggested numerous biochemical molecules such as aspartate aminotransferase, alanine aminotransferase and gamma globulin for cirrhosis or liver fibrosis detection

\[10-13]\). However, most of these proteins were investigated in patients with chronic hepatitis C.

It is reported that chronic inflammation is associated with persistent hepatic injury and concurrent tissue regeneration which leads to sequential development of fibrosis, cirrhosis and eventually HCC

\[11]\). In addition, about one fourth of mild chronic hepatitis patients are affected by cirrhosis within 1 to 13 years of hepatitis onset

\[14]\).

Considering the role of inflammatory responses in cirrhosis development, we designed a research to evaluate diagnostic value of hematological parameters in HBV-related cirrhosis patients compared to healthy people. We focused on white blood cell count (WBC), red blood cell distribution width (RDW), mean platelet volume (MPV), MPV/PC ratio, platelet count/lymphocytes ratio (PLR) and number of neutrophils/lymphocytes ratio (NLR). The corresponding ROC curves were used to evaluate diagnostic value. At last, a model was established for the prediction of HBV-related cirrhosis and was validated in four different validation sets from four different hospitals.

Materials And Methods

**Training set**

We investigated 102 patients with HBV-related cirrhosis and 102 healthy individuals at Zhongnan Hospital from November 2016 to March 2018. The retrospectively study was under approval of Medical Ethics Committee, Zhongnan Hospital of Wuhan University (201707), and written informed consent was obtained from all participants. Their demographic and blood test results were reviewed in the hospital medical database.

**Logistic regression models**

A formula for predicting HBV-related cirrhosis was built based on the data in the Training set. The standard logistic regression formula is:

\[\text{Logit}(P) = \beta_0 + \beta_1X_1 + \beta_2X_2 + \ldots + \beta_nX_n.\]

Regarding \(\text{Logit}(P)=\ln[p/(1 - p)]\), "\(p\)" is the estimated probability of HBV-related cirrhosis, "\(\beta_0\)" is a constant, "\(\beta\)" is the influence coefficient, "\(n\)" is the number of influence factors and "\(X\)" is the influence factors.

**Validation sets**

Four validation sets were recruited from four centers (Zhongnan Hospital of Wuhan University; Tongji Hospital, Tongji Medical College of Huazhong University Science and Technology; Union Hospital, Tongji Medical College of Huazhong Science and Technology University; Renmin Hospital of Wuhan University) and were used to assess the performance of the model. They included 261 HBV-related cirrhosis patients and 288 healthy people. 70 HBV-related cirrhosis patients
and 80 healthy people, 35 HBV-related cirrhosis patients and 45 healthy people, 31 HBV-related cirrhosis patients and 38 healthy people, 125 HBV-related cirrhosis patients and 125 healthy people were recruited in these sets, respectively.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL, USA) or Prism6 (GraphPad software, La Jolla, CA). Data were presented as the mean ± standard deviation (SD). The Shapiro-Wilk test was used to check the normality of the distribution. Normally distributed numeric variables were evaluated by Student’s t-test or one-way analyses of variance (ANOVA). Non-normally distributed variables were analyzed by the Mann-Whitney test or nonparametric test and Chi-square test was used to analyze the categorical variables. A difference was considered statistically significant when \( P < 0.05 \).

Results

Demographic parameters of the training set

129 patients with cirrhosis were recruited in this research. The flow chart of the research was showed in Figure 1. After exclusion of 16 patients with alcoholic cirrhosis and 11 patients with chronic hepatitis C infection, 102 patients with HBV-related cirrhosis and 102 age-matched healthy people were enrolled. The healthy people had no medical record of tumor and matched the HBV-related cirrhosis patients in gender (\( p=0.389 \)), age (\( p=0.572 \)), height (\( p=0.646 \)), weight (\( p=0.190 \)) and smoking (\( p=0.751 \)) (Table S1).

Hematological parameters of the training set

We focused on the expression level of WBC, RDW, MPV, MPV/PC ratio, PLR, and NLR in cirrhosis patients. As presented in Table 1, the expression level of RDW, MPV, MPV/PC ratio, PLR and NLR were all significantly higher in HBV-related cirrhosis patients compared to healthy individuals. No significant difference in WBC was found between HBV-related cirrhosis patients and healthy people. ROC curve analysis showed that the AUC of RDW, MPV, MPV/PC ratio, PLR and NLR were 0.970, 0.772, 0.876, 0.702 and 0.817 in the cirrhosis patient group (Figure 2A). The information of diagnostic value of RDW, MPV, MPV/PC ratio, PLR and NLR was listed in Table S2.

The correlation between clinical parameters and hematological parameters

Chronic inflammation is associated with persistent hepatic injury and concurrent tissue regeneration, leading to sequential development of fibrosis, cirrhosis and eventually Hepatocellular carcinoma. We analyzed the correlation between clinical parameters and hematological parameters to find out the role of these parameters in the progression and metastasis of HBV-related cirrhosis patients. The result showed MPV/PC ratio and PLR were significantly correlated with the Child-Pugh score while other parameters not (Figure 2B to 2F). In addition, all these hematological parameters RDW, MPV, MPV/PC ratio, PLR and NLR were not correlated with smoking or gender (Table 2).

The logistic regression model for HBV-related cirrhosis

The parameters that were significantly changed in the HBV-related cirrhosis patients were then used in the multivariate logistic regression model. RDW, MPV, MPV/PC ratio, PLR and NLR were all considered as independent variables (Table 1) and were used for the building of multivariate model. The data from 102 HBV-related cirrhosis patients and 102 healthy volunteers in the training set was used to build the logistic regression model. The resultant model for predicting HBV-related cirrhosis was:

\[
\text{Logit}(P) = \frac{29.167 - 0.109(MPV) - 1.571(RDW) - 73.438(MPV/PC ratio) - 0.045(NLR) - 0.011(PLR)}{0.045(NLR) - 0.011(PLR)}
\]

The AUC of the model for HBV-related cirrhosis was 0.880, with sensitivity of 80.0% and specificity of 96.7%, respectively (Figure 3A). The ROC curve was measured to evaluate the diagnostic value of each hematological parameter.

Multicenter validation of the model

The validity of the model was assessed in four different validation sets. 261 HBV-related cirrhosis patients (70, 35, 31 and 125 patients from four centers, respectively) and 288 healthy volunteers (80, 45, 38 and 125 volunteers from four centers, respectively) were enrolled for the research.

The estimated probability of 261 HBV-related cirrhosis patients and 228 healthy volunteers were calculated by using the formula Logit (P). In the cohort of Zhongnan Hospital, the probability of 60 (out of 70) HBV-related cirrhosis patients were less than 0.774, and the probability of 70 (out of 80) healthy volunteers were more than 0.774 (Figure 3A). The AUC of the model for HBV-related cirrhosis was 0.866, with sensitivity of 85.7% and specificity of 87.5%, respectively. In the cohort of Tongji Hospital, the probability of 28 (out of 35) HBV-related cirrhosis patients were less than 0.774, and the probability of 42 (out of 45) healthy volunteers were more than 0.774 (Figure 3B). The AUC of the model for HBV-related cirrhosis was 0.880, with sensitivity of 80.0% and specificity of 93.3%, respectively. In the cohort of Union Hospital, the probability of 24 (out of 31) HBV-related cirrhosis patients were less than 0.774, and the probability of 33 (out of 39) healthy volunteers were more than 0.774 (Figure 3C). The AUC of the model for HBV-related cirrhosis was 0.813, with sensitivity of 77.4% and specificity of 84.6%, respectively. In the cohort of Renmin Hospital, the probability 110 (out of 125) HBV-related cirrhosis patients were less than 0.774, and the probability 113 (out of 125) healthy volunteers were more than 0.774 (Figure 3D). The AUC of the model for HBV-related cirrhosis was 0.892, with sensitivity of 88.0% and specificity of 90.4%, respectively. The diagnostic performances of this model were presented in Table 3.
Discussion

Early diagnosis of cirrhosis is important for reducing the complications in patients with chronic HBV infection\textsuperscript{[15,16]}. The liver biopsy is the standard test for distinguishing different liver diseases such as cirrhosis\textsuperscript{[15]} but this test is often associated with high risks of other severe complications, as well as patient discomfort, and costly expense. Therefore, identification a reliable and non-invasive cirrhosis diagnosis method is in high demand by physicians and surgeons.

In the present research, we attempted to build a novel affordable and effective model to predict HBV-related cirrhosis using hematological parameters. The blood test, which is routinely done to the chronic HBV infection patients, has essential implications for the natural history of chronic HBV infection\textsuperscript{[17]}. We evaluated the diagnostic value of hematological parameters including WBC, RDW, MPV, MPV/PC ratio, PLR and NLR. Our results indicate that the expression level of RDW, MPV, MPV/PC ratio, PLR and NLR were significantly increased in HBV-related cirrhosis patients. Most of them were indicative of HBV-related cirrhosis. Among them, RDW owned the best diagnostic value (AUC=0.970) for HBV-related cirrhosis. However, no significant correlation was found between RDW and the Child-Pugh score. RDW, which is used in the differential diagnosis of anemia, is a measure of the range of variation of red blood cell volume. Previous studies have reported that high RDW level was related to the high mortality risk in patients with various disorders\textsuperscript{[18-20]}. In our study, we found that RDW was profoundly higher in HBV-related cirrhosis patients compared to healthy people. Our data is consistent with the previous report\textsuperscript{[21]}. The reasons of the elevated RDW might be vitamin B12 or iron deficiency. Interestingly, the AUC of MPV/PC ratio was lower than RDW, but MPV/PC ratio had the highest specificity for HBV-related cirrhosis, in addition, MPV/PC ratio and PLR were significantly correlated with the Child-Pugh score, while RDW not. Therefore, it would be better that combining multiple parameters to detect HBV-related cirrhosis.

We built the model which combining multiple hematological parameters including RDW, MPV, MPV/PC ratio, PLR and NLR. It had better diagnostic value than any single hematological parameter. Furthermore, we validated the logistic regression model in four different validation sets. We recruited HBV-related cirrhosis patients and healthy volunteers from four different hospitals to constitute different validation sets to avoid the selection bias. The model showed good diagnostic value in all validation sets, indicating that it is effective in predicting HBV-related cirrhosis patients. Previous studies have reported the important role of single biochemical indicators or hematological parameters in cirrhosis diagnosis. Kayadibi\textsuperscript{[22]} showed that cirrhosis might be accurately predicted through measuring platelet count, ALT and AST. Koda M\textsuperscript{[23]} also found that AST to platelet ratio could be a surrogate marker for cirrhosis detection. However, most of these indices were tested in HCV-related cirrhosis patients. In addition, the effectiveness of combinatory inflammatory index in the diagnosis of HBV-related cirrhosis is not well studied. In our research, the model we built showed a better predictive ability than any single inflammatory index. Therefore, this model could be a promising tool for the diagnosis of HBV-related cirrhosis.

Conclusion

This new logistic regression model we built might improve the diagnosis of HBV-related cirrhosis.

Abbreviations

RT-qPCR: Real-time quantitative PCR  PLR: platelet count/lymphocytes ratio

NLR: number of neutrophils/lymphocytes ratio (NLR)

Declarations

Ethics approval and consent to participate

The retrospectively study was under approval of Medical Ethics Committee, Zhongnan Hospital of Wuhan University (201707), and written informed consent was obtained from all participants.

Consent for publication

Not applicable

Acknowledgements

Not applicable

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

Chang-Liang Luo and Zhong-Lin Zhang performed the study; Chang-Liang Luo and Hao Chen analyzed the data; Wu-Wen Zhang, Long Wu, Diao Wei and Xiuhui Qi were responsible for collecting the sample and clinical data; Fu-Bing Wang, Han-Ning Hu and Chang-Liang Luo designed the study and wrote the manuscript; Fu-Bing Wang was responsible for obtaining funding; Chang-Liang Luo and Zhong-Lin Zhang are joint first authors; Fu-Bing Wang and Han-Ning Hu are joint corresponding authors.

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**Tables**

### Table 1 Hematological parameters analysis of Training set

| Parameters         | HBV-related cirrhosis | Healthy controls | P    |
|--------------------|------------------------|------------------|------|
|                   | n=102                  | n=102            |      |
| WBC                | 6.46±3.61              | 6.18±1.26        | 0.115|
| MPV (FL)           | 9.71±1.20              | 8.59±0.97        | < 0.0001|
| RDW(%)             | 17.68±3.64             | 13.07±0.64       | < 0.0001|
| MPV/PC ratio       | 0.086±0.046            | 0.041±0.012      | < 0.0001|
| NLR                | 6.45±7.43              | 1.83±0.73        | < 0.0001|
| PLR                | 229.61±170.06          | 114.99±40.73     | < 0.0001|

Hematological parameters are expressed as mean ± SD; WBC: White blood cell, PC: platelet count, MPV: mean platelet volume, MPV: mean platelet volume, RDW: red blood cell distribution width, MPV/PC ratio: mean platelet volume to platelet count ratio, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio;

### Table 2 Correlation between hematological parameters level and clinical parameters

| Parameters | Group     | n   | MPV   | RDW   | MPV/PC ratio | NLR | PLR   |
|------------|-----------|-----|-------|-------|--------------|-----|-------|
|            |           |     | Mean ±SD | P value | Mean ±SD | P value | Mean ±SD | P value | Mean ±SD | P value |
| Sex        | Male      | 73  | 9.70±1.23 | 0.596 | 17.43±3.15 | 0.899 | 0.085±0.046 | 0.251 | 5.93±6.79 | 0.795 | 220.69±158.73 | 0.750 |
|            | Female    | 29  | 9.72±1.12 | 0.260 | 18.30±4.65 | 0.087±0.047 | 0.069±0.043 | 0.804 | 7.74±8.83 | 0.689 | 252.08±196.99 | 0.477 |
| Smoking    | Negative  | 74  | 9.75±1.18 | 0.793 | 17.81±3.51 | 0.083±0.043 | 0.793±6.71 | 0.69 | 231.14±159.56 | 0.477 |
|            | Positive  | 28  | 9.61±1.25 | 0.549 | 17.35±4.02 | 0.093±0.052 | 0.109±0.051 | 0.001** | 6.94±8.36 | 0.689 | 246.27±162.36 | 0.016* |
| Child-Pugh | A-B       | 74  | 9.74±1.15 | 0.632 | 17.69±3.95 | 0.549 | 0.077±0.041 | 0.001** | 5.16±3.84 | 0.689 | 185.59±184.80 | 0.477 |
|            | C         | 28  | 9.63±1.33 | 0.109±0.051 | 17.66±2.71 | 0.077±0.041 | 0.001** | 6.94±8.36 | 0.689 | 246.27±162.36 | 0.016* |

* p < 0.05, **p < 0.01.

### Table 3 Diagnostic performances of Logistic regression model in training set and validation sets

| Group         | HBV-related cirrhosis patients vs Healthy controls |
|---------------|---------------------------------------------------|
|               | AUC  | 95% CI  | P value | Se (%) | Sp (%) |
| Training set  | Zhongnan Hospital | 0.987 | 0.975- 0.999 | <0.0001 | 96.1 | 95.1 |
| Validation sets | Zhongnan Hospital | 0.866 | 0.803- 0.929 | <0.0001 | 85.7 | 87.5 |
|               | Tongji Hospital | 0.880 | 0.797- 0.963 | <0.0001 | 80.0 | 93.3 |
|               | Union Hospital | 0.813 | 0.705- 0.920 | <0.0001 | 77.4 | 84.6 |
|               | Renmin Hospital | 0.892 | 0.847- 0.937 | <0.0001 | 88.0 | 90.4 |

Se: sensitivity; Sp: specificity

**Figures**
Figure 1

The flow chart of the retrospective study.
Figure 1

The flow chart of the retrospective study.
Figure 2

(A) The ROC curve analysis for the diagnostic value of MPV (AUC = 0.772, 95% CI = 0.708-0.836, p < 0.0001), RDW (AUC = 0.970, 95% CI = 0.949-0.990, p < 0.0001), MPV/PC ratio (AUC = 0.876, 95% CI = 0.822-0.931, p < 0.0001), NLR(AUC = 0.817, 95% CI = 0.755-0.879, p < 0.0001), PLR (AUC = 0.702, 95% CI = 0.626-0.778, p < 0.0001) and model(AUC = 0.987, 95% CI = 0.975-0.999, p < 0.0001) in 102 patients with HBV-related cirrhosis.. (B) MPV/PC ratios of patients with HBV-related cirrhosis in different Child-Pugh scores (p =0.001). (C) PLR of patients with HBV-related cirrhosis in different Child-Pugh scores (p =0.016). (D) MPV of patients with HBV-related cirrhosis in different Child-Pugh scores (p=0.632). (E) RDW of patients with HBV-related cirrhosis in different Child-Pugh scores (p = 0.848). (F) NLR of patients with HBV-related cirrhosis in different Child-Pugh scores (p = 0.848). ns:no significance,* p < 0.05, **p < 0.01.
Figure 3

The scatter diagrams of the logistic regression model in four external validation sets. (A) 10 false negative patients with HBV-related cirrhosis and 10 false positive patients in the cohort of Zhongnan Hospital. (B) 7 false negative patients with HBV-related cirrhosis and 3 false positive patients in the cohort of Tongji Hospital. (C) 7 false negative patients with HBV-related cirrhosis and 6 false positive patients in the cohort of Union Hospital. (D) 15 false negative patients with HBV-related cirrhosis and 12 false positive patients in the cohort of Renmin Hospital.

Supplementary Files

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- TableS1andS2.docx
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