

Development and Validation of a Nomogram for Predicting Severity in Patients With Hemorrhagic Fever With Renal Syndrome: a Retrospective Study

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Research Article

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Abstract

Background: Hemorrhagic fever with renal syndrome (HFRS) is a zoonotic disease caused by hantavirus infection. China is one of the most endemic countries of HFRS in the world. Patients with severe HFRS may develop multiple organ failure or even death, which makes HFRS a serious public health problem in China. Therefore, we constructed and verified a reliable nomogram to predict the severity in patients with HFRS and provide guidance for medical practice.

Methods: In this retrospective study, we included a total of 155 consecutive patients with HFRS who were diagnosed from January 1, 2015 to December 31, 2019, of which 109 patients served as a training cohort and 46 patients as an independent verification cohort. 54 laboratory and clinical indicators were applied to assess the severity of HFRS patients. In the training set, the least absolute shrinkage and selection operator (LASSO) regression was used to screen the characteristic variables of the risk model. Multivariate logistic regression analysis was used to construct a nomogram containing the characteristic variables selected in the LASSO regression model. The nomogram's performance was evaluated by the discrimination, calibration, and clinical applicability in the training set and validation set.

Results: The prediction nomogram included six predictors such as neutrophils, hemoglobin (Hb), platelets, creatinine, calcium (Ca) and dyspnea, which were screened by LASSO regression. The area under the receiver operating characteristic curve (AUC) of the nomogram in the training and validation cohorts was 0.969 (95%CI:0.935-1.000) and 0.934(95%CI: 0.847-1.000), respectively, indicating that the model has good discrimination. The calibration curve exhibited that the nomogram was in good agreement between the prediction and the actual observation. Decision curve analysis and clinical impact curve indicated that the predictive nomogram had clinical utility.

Conclusion: In this study, we established a simple and feasible model to predict severity in patients with HFRS, with which HFRS will be better identified and patients can be treated early.

Background

Hemorrhagic fever with renal syndrome(HFRS)is a rodent-borne zoonotic disease caused by hantavirus infection. HFRS can be caused by *Hantaan virus (HTNV)*, *Dobrava virus (DOBV)*, *Seoul virus (SEOV)*, *Amur virus (AMV)* and *Puumala virus (PUUV)*, etc. The severity of HFRS patients caused by different viral infections is also different ^[1]. HFRS is characterized by systemic vascular endothelial dysfunction and increased vascular permeability. The clinical manifestations include fever, hemorrhage, renal insufficiency, thrombocytopenia and shock ^[2,3]. HFRS is mainly prevalent in Asia and Europe, while China is the most serious epidemic area in the world. A total of 1,118,124 cases were reported during 2008–2018 in China, and every year more than 90% of global HFRS cases were reported ^[4,5,6]. In China, HFRS is mainly infected by *HTNV* and *SEOV*, and the mortality rate of HFRS caused by these viruses is between 5–15%, which makes HFRS a serious public health concern ^[7]. To date, there is no effective antiviral treatment for HFRS, which leads to a high mortality rate in critically ill cases. Therefore, early and

accurate assessment of the severity and prognosis of HFRS patients is of great significance for guiding clinical treatment and the reasonable allocation of medical resources.

However, there is currently no simple and effective model to predict the severity in patients with HFRS. A study shows that the Sequential Organ Failure Assessment (SOFA) score is related to the severity of HFRS, but the scoring system is more complex, does not include the clinical characteristics of patients, and cannot directly reflect the severity of patients, so its clinical application is limited [8]. Nomogram is a statistical prediction model established based on the characteristic phenotype of the disease, which is used to predict the probability of a certain outcome event in a population with certain characteristics in the future. Nomogram transforms the complex regression equation into a visual graph, making the results of the prediction model more readable and convenient to evaluate the patient's condition [9]. With this clinical prediction model, doctors can simply and accurately predict the patient's condition, thereby providing a basis for clinical decision-making. Consequently, in this study, we retrospectively analyzed the clinical characteristics and laboratory results of HFRS patients, and aimed to develop and verify a simple and applicable nomogram that predicts the severity of the patient's condition. It will be the first nomogram of HFRS.

Methods

Study Population

This study retrospectively analyzed a total of 155 consecutive patients with HFRS diagnosed in Jingzhou Central Hospital from January 1, 2015 to December 31, 2019. 109 patients from January 1, 2015 to December 31, 2018 served as a training cohort, and 46 patients from January 1, 2019 to December 31, 2019 as an independent verification cohort. Patients with confirmed HFRS were included in the study. The diagnostic criteria of the patients were as follows: 1) acute fever, accompanied by abnormal renal function, thrombocytopenia, etc.; and 2) the hantavirus-specific immunoglobulin (Ig) M antibody in the peripheral blood is positive. The exclusion criteria included: 1) age < 18 years old; 2) pregnant women; 3) acute or chronic nephropathy and hematological diseases.

Data collection

Well-trained doctors extracted the patient's demographic characteristics, basic diseases, clinical manifestations, and laboratory parameters through the electronic medical record system. Laboratory parameters included complete blood count, urine routine, procalcitonin (PCT), C-reactive protein (CRP), liver and kidney function, electrolytes, myocardial enzymes, and hantavirus-specific antibodies.

According to the clinical characteristics of patients, such as body temperature, blood pressure, urine output, edema and renal injury indicators such as urinary protein and urea nitrogen, the severity of HFRS was divided into four clinical types [10]. The four clinical types are as follows: 1) the mild group has renal injury without hypotension and oliguria; 2) the moderate group has obvious uremia, bulbar conjunctival edema, skin and mucosal hemorrhage, and acute renal failure with typical oliguria; 3) the severe group

showed severe uremia, bulbar conjunctiva and peritoneal or pleural effusion, skin and mucosal bleeding, hypotension and acute renal failure with oliguria (patients with daily output of 50 to 500 mL \leq 5 days or urine output < 100mL/day \leq 2 days); 4) The critically ill group has one or more of the following manifestations compared with the severe group: refractory shock (\geq 2 days), heart failure, pulmonary edema, visceral hemorrhage, cerebral edema, severe secondary infection and severe acute renal failure with oliguria (urine volume 50-500 mL/day > 5 days) or anuria (urine < 100 mL/day > 2 days) or blood urea nitrogen (BUN) > 42.84 mmol/L. In this study, patients were divided into two groups, the mild group was composed of mild and moderate patients, and the severe group was composed of severe and critically ill patients.

Ethics statement

The study was approved by the Ethics Committee of Jingzhou Central Hospital, and informed consent was abandoned because the study was a retrospective study.

Statistical analysis

All statistical analyses in this study were carried out using R software (version 4.0.3; <http://www.r-project.org>). The statistical significance levels of all reports were double-tailed, and $p < 0.05$ was considered statistically significant. The R software packages involved in the implementation of R software mainly include compareGroups, glmnet, rms, pROC, rmda and so on. The demographic characteristics, basic diseases, clinical manifestations and laboratory parameters were statistically analyzed by compareGroups R software package, in which the Shapiro-Wilks test was performed to determine whether it was normal or non-normal distribution. Continuous variables with a normal distribution were expressed as the mean \pm standard deviation (SD) while non-normally distributed continuous variables were expressed as the median (interquartile range). Categorical variables were presented as percentages (%). LASSO regression is a model in which the L1-norm constraint term is added to the cost function of the linear regression model. It is used to analyze medical data with high dimension, strong correlation and small samples by controlling the parameter lambda for variable screening and complexity adjustment^[11]. In this study, the glmnet package in LASSO regression was used to select the best predictive characteristics of risk factors from HFRS patients. Multivariate logistic regression analysis was applied to construct the nomogram of the predictive model by including the selected variables with non-zero coefficient characteristics in the LASSO regression model^[12].

We evaluated the performance of the nomogram through discrimination and calibration in the training population and the verification population, respectively. Since the consistency index (C-index) is equivalent to the area under the receiver operating characteristic curve (AUC) in logistic regression, we use the AUC to evaluate the discriminative ability of the nomogram^[13]. The Hosmer-Lemeshow goodness-of-fit test is used to evaluate the calibration of the nomogram, and a calibration curve is drawn to visualize the consistency between the predicted results and the observed results^[14]. By quantifying the net benefit under each risk threshold probability, the decision curve analysis (DCA) of the model is drawn to evaluate

the clinical validity of the nomogram ^[15]. We drew a nomogram plot and a calibration plot based on the rms R package. The pROC R package was used to draw the receiver operating characteristic (ROC) curve and calculate the C-index. The rmda R package was used to draw the DCA and clinical impact curve.

Results

Demographic and clinical characteristics of patients with HFRS

A total of 155 HFRS patients were included in our study, of which 11 died, with a mortality rate of 7.10%. Table 1 summarizes the demographic characteristics of HFRS in the training cohort and the verification cohort, showing that there is no significant difference in gender, age, basic disease, clinical disease classification, and clinical outcome between the two populations. We analyzed the clinical characteristics of mild and severe groups in the training cohort of 109 patients with HFRS. The median age of the training cohort was 53 years old, including 79 males and 30 females (Table 2). The most common clinical manifestations of HFRS patients were fever (90.8%), oliguria (58.7%), nausea (35.8%), chills (35.8%), vomiting (33.0%), diarrhea (28.4%), headache (26.6%), low back pain (25.7%), fatigue (22.0%), abdominal distension (20.2%) and so on. Among the above symptoms, only oliguria and arthralgia were statistically different between the critically ill group and the mild group. The results of laboratory examination showed that the levels of white blood cell (WBC), neutrophils, lymphocytes, procalcitonin (PCT), C-reactive protein (CRP), urine protein, urea nitrogen, creatinine, cystatin C, creatine kinase, creatine kinase muscle-brain isoform (CK-MB) and myoglobin increased more significantly in severe HFRS patients, while the levels of platelets (PLT), hemoglobin (Hb), albumin and calcium (Ca) decreased more significantly in severe patients.

Table 1
Baseline Characteristics of patients with HFERS in the training and validation cohorts

Characteristic	All patient	Training Cohort	Validation Cohort	<i>P</i> value
	N = 155	N = 109	N = 46	
sex				0.790
female	41 (26.5%)	30 (27.5%)	11 (23.9%)	
male	114 (73.5%)	79 (72.5%)	35 (76.1%)	
Age, years	54.0 (47.0,62.0)	53.0 (47.0,62.0)	55.0 (50.0,63.8)	0.323
Basic disease				0.139
no	109 (70.3%)	81 (74.3%)	28 (60.9%)	
yes	46 (29.7%)	28 (25.7%)	18 (39.1%)	
Clinical type				0.474
mild	69 (44.5%)	46 (42.2%)	23 (50.0%)	
severe	86 (55.5%)	63 (57.8%)	23 (50.0%)	
Clinical outcomes				0.508
Deceased	11 (7.10%)	9 (8.26%)	2 (4.35%)	
Survived	144 (92.9%)	100 (91.7%)	44 (95.7%)	
Basic diseases include hypertension, diabetes, coronary heart disease, stroke, chronic liver disease, chronic lung disease and other diseases. The <i>p</i> values indicate differences between training and validation cohorts. <i>P</i> < 0.05 was considered statistically significant.				

Table 2
Demographic and clinical features of patients with HFRS in the training cohorts

Characteristic	All patient	mild	severe	<i>P</i> value
	N = 109	N = 46	N = 63	
Sex				0.218
female	30 (27.5%)	16 (34.8%)	14 (22.2%)	
male	79 (72.5%)	30 (65.2%)	49 (77.8%)	
Age, years	53.0 (47.0,62.0)	50.5 (47.0,62.0)	57.0 (46.5,62.5)	0.337
Signs and symptoms				
Fever				0.186
no	10 (9.17%)	2 (4.35%)	8 (12.7%)	
yes	99 (90.8%)	44 (95.7%)	55 (87.3%)	
Chills				0.428
no	70 (64.2%)	32 (69.6%)	38 (60.3%)	
yes	39 (35.8%)	14 (30.4%)	25 (39.7%)	
Headache				1.000
no	80 (73.4%)	34 (73.9%)	46 (73.0%)	
yes	29 (26.6%)	12 (26.1%)	17 (27.0%)	
Nausea				1.000
no	70 (64.2%)	30 (65.2%)	40 (63.5%)	
yes	39 (35.8%)	16 (34.8%)	23 (36.5%)	
Vomiting				0.053
no	73 (67.0%)	36 (78.3%)	37 (58.7%)	
yes	36 (33.0%)	10 (21.7%)	26 (41.3%)	
Abdominal bloating				0.917
no	87 (79.8%)	36 (78.3%)	51 (81.0%)	

The *p* values indicate differences between mild and severe groups. $P < 0.05$ was considered statistically significant. Abbreviations: WBC, white blood cell; Hb, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; Ca, calcium; K, potassium; P, phosphorus; CK-MB, creatine kinase muscle-brain isoform; cTnI, cardiac troponin I.

Characteristic	All patient	mild	severe	<i>P</i> value
yes	22 (20.2%)	10 (21.7%)	12 (19.0%)	
Poor appetite				0.356
no	95 (87.2%)	38 (82.6%)	57 (90.5%)	
yes	14 (12.8%)	8 (17.4%)	6 (9.52%)	
Abdominal pain				0.731
no	100 (91.7%)	43 (93.5%)	57 (90.5%)	
yes	9 (8.26%)	3 (6.52%)	6 (9.52%)	
Backache				0.762
no	81 (74.3%)	33 (71.7%)	48 (76.2%)	
yes	28 (25.7%)	13 (28.3%)	15 (23.8%)	
Diarrhea				0.124
no	78 (71.6%)	37 (80.4%)	41 (65.1%)	
yes	31 (28.4%)	9 (19.6%)	22 (34.9%)	
Dyspnea				0.072
no	104 (95.4%)	46 (100%)	58 (92.1%)	
yes	5 (4.59%)	0 (0.00%)	5 (7.94%)	
Oliguria				0.010
no	45 (41.3%)	26 (56.5%)	19 (30.2%)	
yes	64 (58.7%)	20 (43.5%)	44 (69.8%)	
Cough				0.731
no	100 (91.7%)	43 (93.5%)	57 (90.5%)	
yes	9 (8.26%)	3 (6.52%)	6 (9.52%)	
Expectoration				1.000
no	104 (95.4%)	44 (95.7%)	60 (95.2%)	

The *p* values indicate differences between mild and severe groups. $P < 0.05$ was considered statistically significant. Abbreviations: WBC, white blood cell; Hb, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; Ca, calcium; K, potassium; P, phosphorus; CK-MB, creatine kinase muscle-brain isoform; cTnI, cardiac troponin I.

Characteristic	All patient	mild	severe	<i>P</i> value
yes	5 (4.59%)	2 (4.35%)	3 (4.76%)	
Chest tightness				0.394
no	104 (95.4%)	45 (97.8%)	59 (93.7%)	
yes	5 (4.59%)	1 (2.17%)	4 (6.35%)	
Black stool				1.000
no	106 (97.2%)	45 (97.8%)	61 (96.8%)	
yes	3 (2.75%)	1 (2.17%)	2 (3.17%)	
Fatigue				0.521
no	85 (78.0%)	34 (73.9%)	51 (81.0%)	
yes	24 (22.0%)	12 (26.1%)	12 (19.0%)	
Orbita Pain				0.261
no	106 (97.2%)	46 (100%)	60 (95.2%)	
yes	3 (2.75%)	0 (0.00%)	3 (4.76%)	
Myalgia				0.163
no	100 (91.7%)	40 (87.0%)	60 (95.2%)	
yes	9 (8.26%)	6 (13.0%)	3 (4.76%)	
Arthralgia				0.029
no	105 (96.3%)	42 (91.3%)	63 (100%)	
yes	4 (3.67%)	4 (8.70%)	0 (0.00%)	
Pulmonary hemorrhage				0.508
no	107 (98.2%)	46 (100%)	61 (96.8%)	
yes	2 (1.83%)	0 (0.00%)	2 (3.17%)	
Gastrointestinal bleeding				0.072
no	104 (95.4%)	46 (100%)	58 (92.1%)	

The *p* values indicate differences between mild and severe groups. $P < 0.05$ was considered statistically significant. Abbreviations: WBC, white blood cell; Hb, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; Ca, calcium; K, potassium; P, phosphorus; CK-MB, creatine kinase muscle-brain isoform; cTnI, cardiac troponin I.

Characteristic	All patient	mild	severe	<i>P</i> value
yes	5 (4.59%)	0 (0.00%)	5 (7.94%)	
Cerebral hemorrhage				0.508
no	107 (98.2%)	46 (100%)	61 (96.8%)	
yes	2 (1.83%)	0 (0.00%)	2 (3.17%)	
History of rat exposure				0.507
no	33 (30.3%)	16 (34.8%)	17 (27.0%)	
yes	76 (69.7%)	30 (65.2%)	46 (73.0%)	
Highest temperature, °C	39.0 ± 0.63	39.1 ± 0.59	39.0 ± 0.65	0.192
Time from symptom onset to admission	5.00 (4.00,7.00)	5.00 (4.00,7.00)	5.00 (4.00,6.00)	0.173
Laboratory findings				
WBC, ×10 ⁹ /L	20.5 (12.4,30.6)	12.6 (9.53,21.5)	25.2 (17.5,35.8)	<0.001
Neutrophils, × 10 ⁹ /L	9.98 (6.41,18.7)	6.52 (4.58,10.0)	14.3 (9.30,22.2)	<0.001
Lymphocytes, × 10 ⁹ /L	5.54 (3.69,8.13)	4.78 (3.07,6.75)	6.30 (4.06,9.25)	0.021
Hb, g/L	107 ± 20.1	115 ± 16.0	100 ± 20.5	<0.001
Platelets, × 10 ⁹ /L	32.0 (15.0,59.0)	54.0 (35.2,93.0)	22.0 (12.0,35.0)	<0.001
Atypical lymphocyte, %	7.50 ± 5.67	6.67 ± 4.39	8.10 ± 6.42	0.173
PCT, ng/mL	3.12 (1.00,7.46)	1.21 (0.60,2.15)	6.16 (2.26,10.7)	<0.001
CRP, mg/L	42.9 (23.5,56.0)	30.9 (19.2,49.9)	51.0 (32.3,73.6)	0.001
Urine protein				0.002
1+	12 (11.0%)	8 (17.4%)	4 (6.35%)	
2+	34 (31.2%)	18 (39.1%)	16 (25.4%)	

The *p* values indicate differences between mild and severe groups. *P* < 0.05 was considered statistically significant. Abbreviations: WBC, white blood cell; Hb, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; Ca, calcium; K, potassium; P, phosphorus; CK-MB, creatine kinase muscle-brain isoform; cTnI, cardiac troponin I.

Characteristic	All patient	mild	severe	<i>P</i> value
3+	44 (40.4%)	19 (41.3%)	25 (39.7%)	
4+	19 (17.4%)	1 (2.17%)	18 (28.6%)	
Albumin, g/L	26.8 (23.8,29.8)	27.9 (24.8,31.5)	25.7 (23.2,28.9)	0.022
ALT, U/L	62.9 (41.7,108)	59.2 (43.0,110)	72.2 (41.7,106)	0.556
AST, U/L	104 (67.7,184)	82.7 (58.2,158)	115 (77.9,222)	0.051
TBIL, μ mol/L	13.9 (10.8,19.0)	12.9 (10.8,17.0)	14.7 (11.2,23.9)	0.114
DBIL, μ mol/L	5.30 (3.80,8.00)	4.70 (3.70,5.77)	6.20 (4.20,10.5)	0.006
Urea nitrogen, mmol/L	21.8 (14.4,28.5)	14.2 (10.1,18.7)	26.7 (21.9,31.6)	<0.001
Creatinine, μ mol/L	483 (220,616)	215 (142,309)	604 (514,723)	<0.001
Uric acid, μ mol/L	596 (485,713)	616 (498,699)	594 (484,744)	0.927
Cystatin C, mg/L	3.73 (2.32,4.53)	2.32 (1.81,3.11)	4.37 (3.72,6.12)	<0.001
Ca, mmol/L	1.72 \pm 0.21	1.85 \pm 0.16	1.62 \pm 0.19	<0.001
K, mmol/L	4.65 \pm 0.70	4.27 \pm 0.57	4.94 \pm 0.65	<0.001
P, mmol/L	0.75 (0.46,0.98)	0.89 (0.74,1.06)	0.53 (0.38,0.83)	<0.001
Creatine Kinase, U/L	184 (87.8,376)	111 (69.4,206)	211 (124,408)	0.002
CK-MB, U/L	40.4 (24.7,54.6)	33.1 (18.3,50.0)	45.1 (27.9,71.2)	0.001
cTnl, μ g/L	0.05 (0.01,0.31)	0.02 (0.01,0.80)	0.06 (0.02,0.21)	0.085
Myoglobin, μ g/L	166 (58.9,289)	65.3 (47.7,281)	236 (92.2,377)	<0.001

The *p* values indicate differences between mild and severe groups. *P* < 0.05 was considered statistically significant. Abbreviations: WBC, white blood cell; Hb, hemoglobin; PCT, procalcitonin; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; DBIL, direct bilirubin; Ca, calcium; K, potassium; P, phosphorus; CK-MB, creatine kinase muscle-brain isoform; cTnl, cardiac troponin I.

Prognostic factors in patients with severe HFRS

After excluding variables with irrelevant characteristics from the training cohort, 54 variables were finally included in the LASSO regression for analysis (Fig. 1A). The parameter lambda (λ) was selected by using 10-fold cross-validation based on the minimum standard in the LASSO model. The two vertical dashed lines in Fig. 1B represent the log (λ) of the minimum mean square error (left dashed line) and the log(λ) of the minimum distance standard error (right dashed line). In order to provide a simple and accurate clinical model, six variables corresponding to the log (λ) of minimum mean square error: "Neutrophils", "Hb", "Platelets", "Creatinine", "Ca" and "Dyspnea" were selected into the model (Fig. 2, Table 3).

Table 3
Prognostic factors in patients with severe HFRS

Intercept and variable	β	Odds Ratio (95% CI)	<i>P</i> value
Intercept	4.437	84.523(0.001, 3.508e + 07)	0.465
Neutrophils	0.013	1.013(0.913, 1.139)	0.811
Hb	-0.037	0.963(0.916, 1.004)	0.103
Platelets	-0.009	0.991 (0.965, 1.0140)	0.481
Creatinine	0.011	1.011(1.007, 1.017)	0.001
Ca	-2.632	0.072(0.000, 16.208)	0.361
Dyspnea	18.937	1.676e + 08(0.000, NA)	0.994

Abbreviations: HFRS, hemorrhagic fever with renal syndrome; β , regression coefficient; CI, confidence interval; Hb, hemoglobin; Ca, calcium; NA, not applicable.

Development and verification of a nomogram

The regression model based on six independent variables for predicting the severity of HFRS determined by LASSO regression analysis was represented by a nomogram (Fig. 2). According to the nomogram, we can get the points corresponding to each predictor, and then record the total score of these points, so as to accurately predict the risk of serious illness in the corresponding HFRS patients. As can be seen in Figs. 3A and Figs. 3B, the AUC of the nomogram in the training and validation cohorts was 0.969 (95%CI:0.935-1.000) and 0.934(95%CI: 0.847-1.000), respectively. The AUC values of the above two are both more than 0.9, indicating that the model has good discrimination. In the training cohort and validation cohort, the calibration plot and Hosmer-Lemeshow goodness-of-fit test showed that the *P* values were 0.745 and 0.398, respectively; both *P* values were > 0.05, indicating that the predicted probability of nomogram was in good agreement with the real results (Figs. 4A, 4B).

Clinical Utility

DCA shows that using nomogram to predict the risk of severe illness in HFRS patients can benefit patients if the threshold probability of the patient or doctor is between 0 and 1 (Fig. 5A). Within this range, according to the nomogram, the net benefit is comparable, but there are multiple overlaps.

Discussion

HFRS is an infectious disease of global concern caused by hantavirus infection, which is characterized by increased vascular permeability, acute thrombocytopenia and renal damage. China is one of the most popular countries in the world [3]. HFRS patients can be clinically manifested as mild, moderate, severe, and critical. Generally, HFRS caused by *HTNV* and *SEOV* infection is more serious, with a mortality rate of 5–15% [7]. The purpose of this study is to analyze the clinical characteristics and laboratory examination of patients with HFRS, so as to establish a nomogram to predict the severity of the disease. Through this simple and feasible prediction model, we can identify the patient's condition early and provide patients with better medical measures in a timely manner to reduce patient mortality.

The typical course of HFRS can be divided into five different stages: fever, hypotension, oliguria, polyuria and recovery. In the hypotension stage, 1/3 of the deaths of HFRS patients are related to irreversible shock, while thrombocytopenia and leukocytosis are the characteristics of this stage. Thrombocytopenia can cause petechiae of the skin or mucous membranes, conjunctival congestion, hematemesis, hemoptysis, hematuria, and fatal intracranial hemorrhage [16]. In addition, platelet dysfunction may also lead to abnormal blood coagulation [17]. In the training cohort of Table 2, there were 63 seriously ill patients, including 2 patients with pulmonary hemorrhage, 5 patients with gastrointestinal hemorrhage and 2 patients with intracranial hemorrhage. However, there is no statistical difference between severe and mild patients due to the small sample size observed.

In this study, the platelets count decreased more significantly in the severe group. At the same time, after the parameter λ was selected by the 10-fold cross-validation based on the minimum standard in the LASSO model, the platelets count was also included in the regression model, indicating that platelets count can be used as a predictor of the severity of HFRS patients.

In patients with viral hemorrhagic fever, platelets can cause abnormal homeostasis and inflammatory activation, thereby inhibiting the body's antiviral immune response, and making patients show a high level of viremia. This mechanism leads to the aggravation of the patient's condition [18]. Other studies have shown that WBC, PLT, platelet distribution width (PDW) and PCT can be used as valuable parameters for the severity of HFRS patients, especially the change of PDW on the first day of hospitalization is related to the survival rate of severe HFRS patients and can be used as potential predictors [19]. In this study, the increase of WBC in patients with severe HFRS was significantly higher than that in mild patients, whereas a study showed that compared with leukocytosis, thrombocytopenia may better predict the prognosis of severe acute kidney injury (AKI) in patients with acute *HTNV* infection [20]. Neutrophil activation is usually common in bacterial infections. It is interesting to note that markers of neutrophil activation, such as myeloperoxidase (MPO), human neutrophil elastase (HNE), histone and

interleukin-8 (IL-8), are significantly increased in the blood and tissue of patients with severe HFRS. These results suggest that neutrophils can be activated by endothelial cells infected by hantavirus and may help to determine the degree of renal pathological damage in patients with severe HFRS [21]. In our study, neutrophil in patients with severe HFRS was also higher than that in mild patients, which may further support this view from a clinical perspective.

Acute renal failure can occur in patients with severe HFRS, usually caused by tubulointerstitial and glomerular damage [22]. In addition, the increase of platelet production and platelet activation may cause intravascular coagulation, the accumulation of inflammatory cells, and the release of pro-inflammatory cytokines in the kidney tissue, which can also lead to kidney damage [23, 24]. In this study, renal function impairment indicators such as urine protein, urea nitrogen, creatinine, and cystatin C were significantly increased in severe HFRS patients. Previous studies have also confirmed that plasma cystatin C and alpha-1-microglobulin (A1M) can be used as early and sensitive markers of renal injury in patients with HFRS, and can predict AKI [25, 26]. The complexity adjustment of LASSO regression model is controlled by the parameter λ , so as to avoid over-fitting. The larger the λ , the greater the penalty for a linear model with more variables, and a model with fewer variables is finally obtained [11], so in the end only creatinine is included in the prediction model. Patients with acute renal failure are often accompanied by hypocalcemia. Wang B [27] studied the prognostic ability of serum calcium in patients with severe AKI, and the results showed that low Ca concentration was an independent predictor of all-cause mortality in patients with severe AKI. Similarly, in our study, the average serum calcium concentration in HFRS patients was lower than normal level, especially in severely ill patients.

In addition, patients with HFRS can also experience acute cardiovascular events such as acute myocardial infarction and stroke, indicating that the increased levels of myocardial injury indicators such as creatine kinase, CK-MB and myoglobin can predict the risk of disease progression in patients [28]. Another study showed that hypoproteinemia in patients with acute HFRS is associated with the severity of the patient's disease, which is consistent with our findings [29]. The clinical manifestations of HFRS patients are diverse, including fever, headache, fatigue, myalgia, back pain and so on [30]. In addition to the above symptoms in this study, gastrointestinal symptoms such as nausea, vomiting, diarrhea, abdominal distension and respiratory symptoms for example cough and dyspnea were also manifested. Severe HFRS patients may initially present as dry cough, followed by tachycardia, dyspnea, and then may rapidly progress to non-cardiogenic pulmonary edema, hypotension and circulatory failure, with a case-fatality rate of about 45% [31].

Based on LOSSA regression, we finally included six predictive indicators: "Neutrophils", "Hb", "Platelets", "Creatinine", "Ca" and "Dyspnea" to establish a nomogram. The AUC value of the nomogram is greater than 0.9 in both the training cohort and the verification cohort, indicating that the predictive model has a high value. Both the calibration plot and Hosmer-Lemeshow goodness-of-fit test show that the prediction probability of nomogram is in good agreement with the real results. In addition, in order to evaluate the clinical effectiveness of nomogram, we apply DCA to provide observations of clinical results based on

threshold probability, from which net benefits can be derived (Net benefit is defined as the proportion of true positives minus the proportion of false positives, weighted by the relative harm of false positive and false negative results) [15, 32]. In this study, if the threshold probability of the patient or doctor is between 0 and 1, the use of nomogram to assess the risk of severe illness in HFRS patients can benefit patients. Clinical impact curve also intuitively shows that the nomogram has a better overall net benefit within a wide range of threshold probability and affecting the prognosis of patients.

However, our research also has some limitations. First, it is designed to be retrospective, and the inherent limitations of this type of research inevitably affect the choice of patients. Second, although we collected patient data from different periods of time to validate the model, it came from a single center. If possible, we still need cohorts from other research centers to validate the model. Finally, the number of cases in our study is relatively small, which may weaken the predictive ability of the current model.

Conclusions

This study developed and verified a novel nomogram for predicting the condition of patients with HFRS, which is the first nomogram used to predict HFRS. Based on these six laboratory and clinical parameters, clinicians can easily and accurately assess the individual risk of HFRS patients, make correct clinical decisions, and provide the best treatment for patients.

Abbreviations

HFRS: hemorrhagic fever with renal syndrome; LASSO: least absolute shrinkage and selection operator; Hb: hemoglobin; Ca: calcium; AUC: area under the receiver operating characteristic curve; CI: confidence interval; *HTNV: Hantaan virus*; *DOBV: Dobrava virus*; *SEOV: Seoul virus*; *AMV: Amur virus*; *PUUV: Puumala virus*; SOFA: sequential organ failure assessment; Ig: immunoglobulin; PCT: procalcitonin; CRP: C-reactive protein; BUN: blood urea nitrogen; SD: standard deviation; C-index: consistency index; DCA: decision curve analysis; ROC: receiver operating characteristic; WBC: white blood cell; CK-MB: creatine kinase muscle-brain isoform; PLT: platelet; λ : lambda; PDW: platelet distribution width; AKI: acute kidney injury; MPO: myeloperoxidase; HNE: human neutrophil elastase; IL-8: interleukin-8; A1M: alpha-1-microglobulin

Declarations

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Authors' contribution

Zheng Yang, Qinming Hu and Yi Sun conceived the study idea, and performed interpretation, manuscript writing and final approval. Zhipeng Feng performed data analysis and collection. All authors reviewed

and approved the final version of the manuscript.

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Availability of supporting data

Due to the finalization of the clinical study report, the data analyzed during the current study are not publicly available, but can be obtained from the correspondent author under reasonable request.

Ethics approval and consent to participate

The study was reviewed and approved for publication by the Institutional Review Board of Jingzhou Central Hospital, and the requirement for informed consent from the study participants was waived.

Consent for publication

Not applicable

Competing interests

The authors declare that there is no conflict of interest.

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Figures

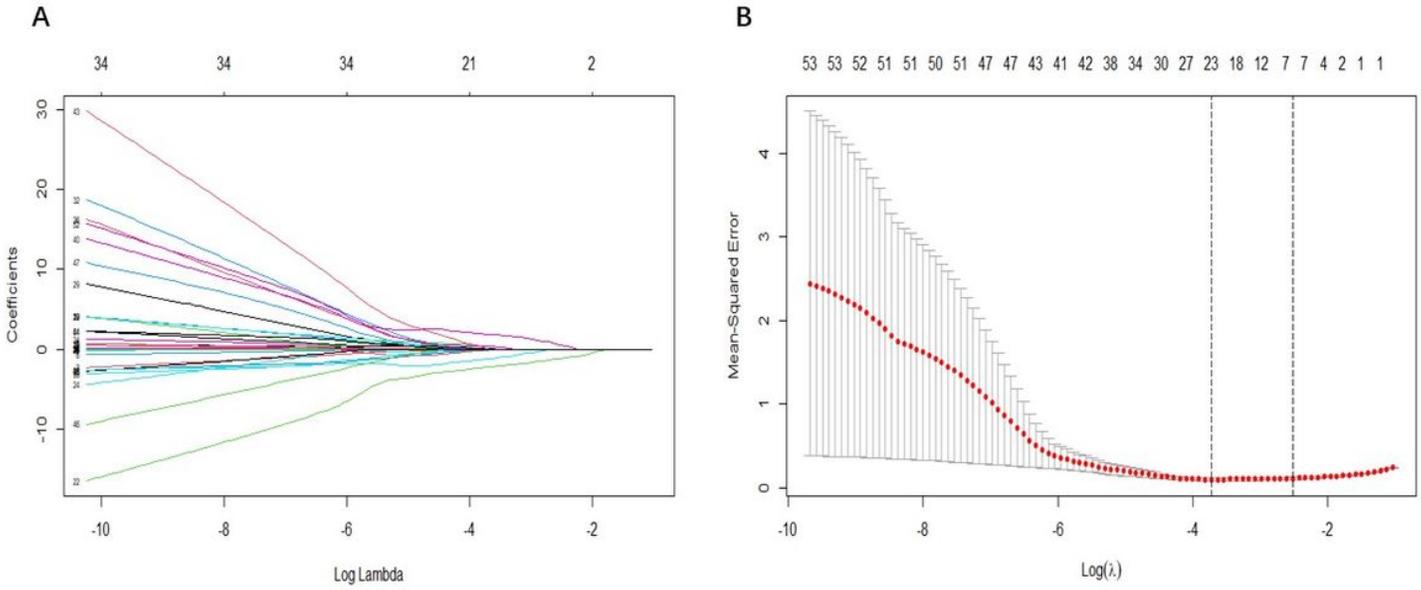


Figure 1

Predictive factors for patients with severe HFRS were selected by LASSO regression. (A) 54 variables from the training cohort were included in the LASSO regression. (B) The parameter lambda (λ) was selected by using 10-fold cross-validation based on the minimum standard in the LASSO model. The two vertical dashed lines represent the log (λ) of the minimum mean square error (left dashed line) and the log(λ) of the minimum distance standard error (right dashed line). Abbreviations: HFRS, hemorrhagic fever with renal syndrome; LASSO, least absolute shrinkage and selection operator; λ , lambda.

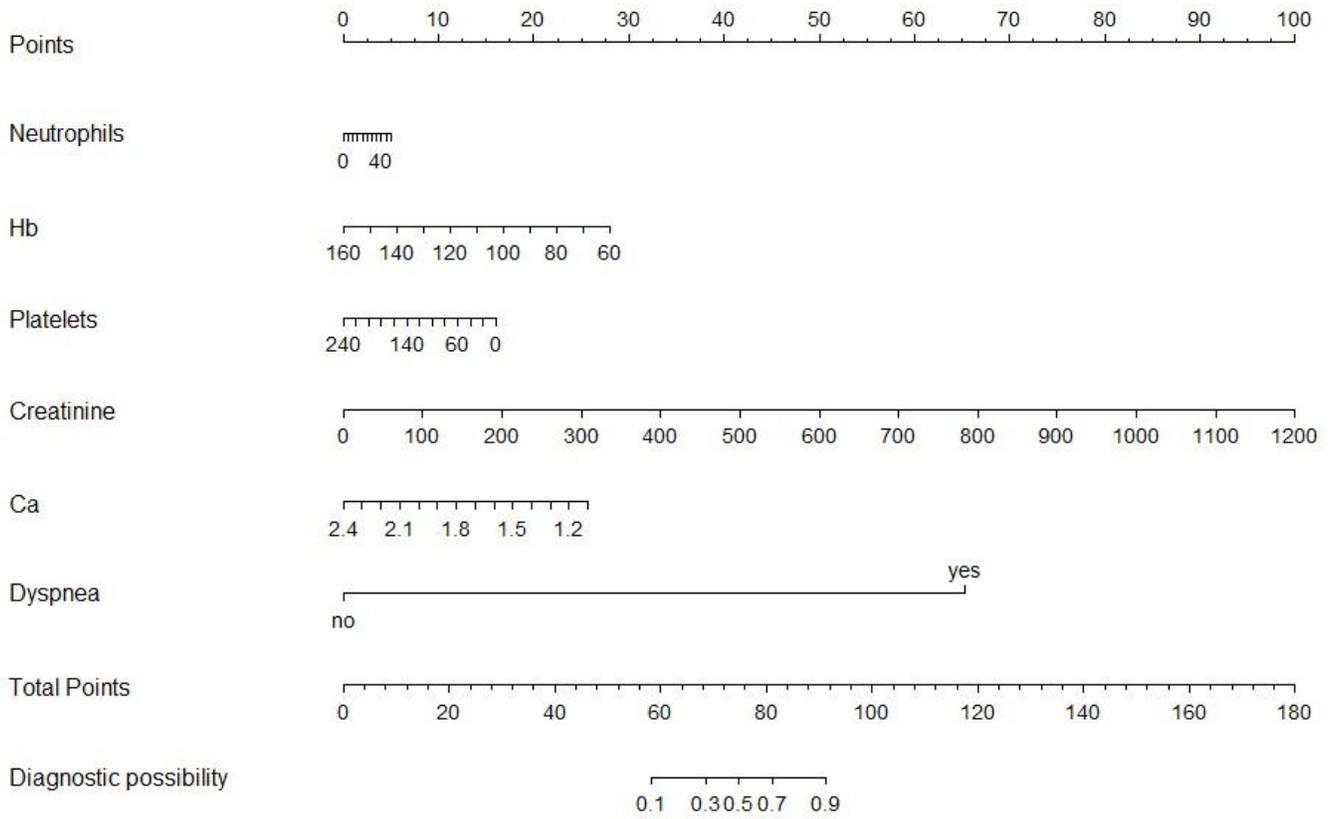


Figure 2

Nomogram to predict the risk of severity in patients with HFRS. Abbreviations: HFRS, hemorrhagic fever with renal syndrome; Hb, hemoglobin; Ca, calcium.

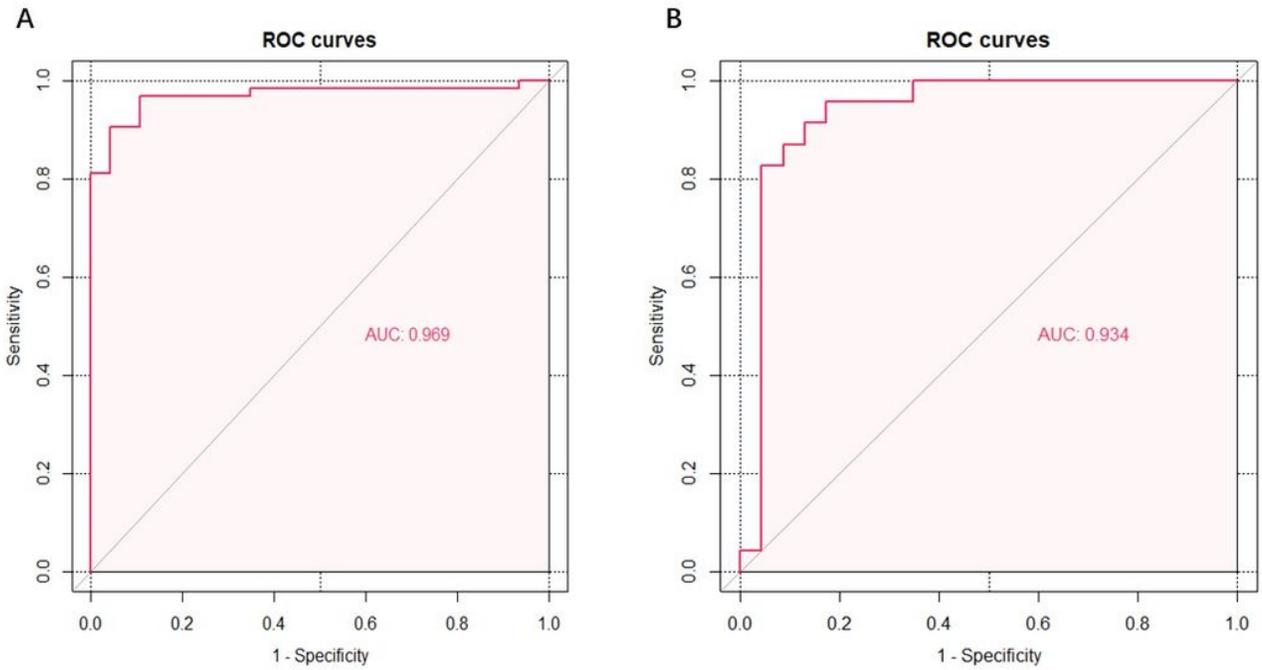


Figure 3

ROC curve to evaluate the discriminative performance of the nomogram in the training and validation cohorts. (A) Training cohort. (B) Validation cohort. Abbreviations: ROC, receiver operating characteristic.

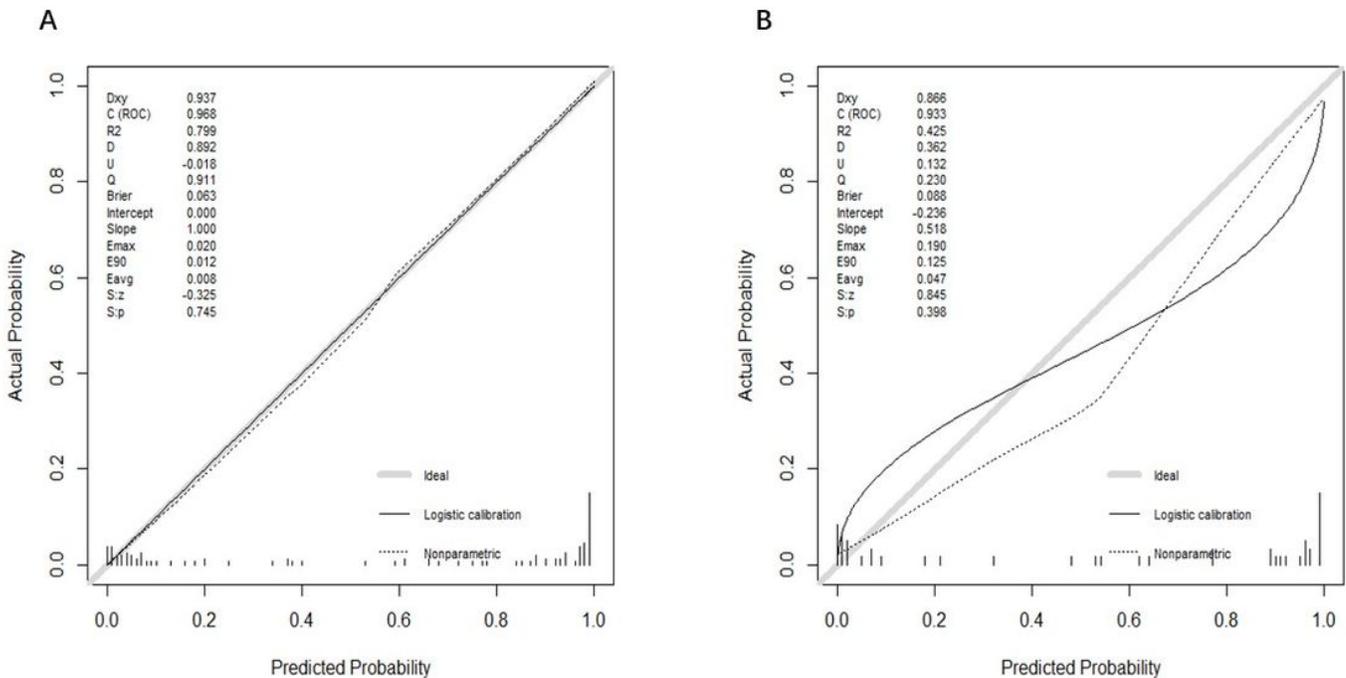


Figure 4

Calibration curves for training and validation of the nomogram. (A) Training cohort. (B) Validation cohort.

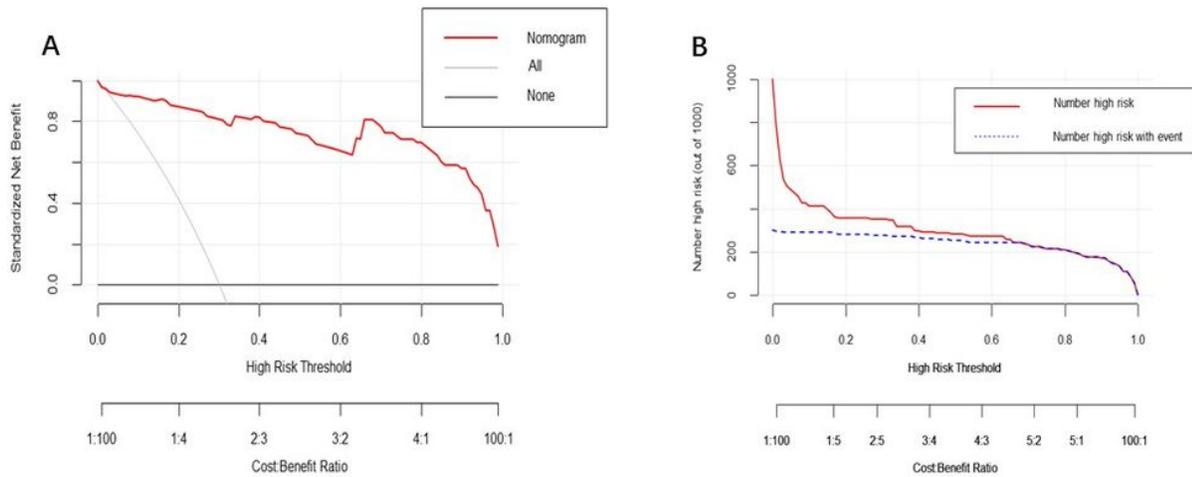


Figure 5

The decision curves and clinical impact curves analysis of the nomogram for predicting severe HFRS. (A) The DCA compares the clinical net benefits of scenarios that predict the probability of severe HFRS: a perfect predictive model (solid grey line), no screening (horizontal solid black line), and screening based on the nomogram (solid red line). (B) Clinical impact curve of the nomogram plots the number of HFRS patients classified as high risk, and the number of cases classified as high risk with the event at each risk threshold. Abbreviations: HFRS, hemorrhagic fever with renal syndrome; DCA, decision curve analysis.