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RESEARCH ARTICLE

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Construction of a predictive model for relapse of primary autoimmune hemolytic anemia: a retrospective cohort study

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ABSTRACT

Objectives: To develop a machine learning-based model to predict the relapse risk of Primary Autoimmune Haemolytic Anaemia (AIHA) after the last remission.

Methods: A retrospective study was conducted on primary AIHA cases who visited the Affiliated Hospital of Southwest Medical University and Xuyong County People's Hospital from May 2017 to May 2022. Cases were categorized as relapsed or non-relapsed based on the 1-year outcomes. Twenty-two features were analyzed to identify relapse risk factors. The least absolute shrinkage and selection operator (LASSO) regression model and multivariate logistic regression analysis were used to establish a predictive model. The *C*-index, Calibration curves, ROC, and Decision curve analysis (DCA) were used to evaluate the discriminatory, corrective, accurate, and clinical effectiveness of the predictive model.

Results: A total of 232 cases of primary AIHA were included, and five potential variables including 'DAT results', 'Hb', 'Multiline therapy', 'Complicating ITP', and 'Complicating infection', have been screened for constructing a 1-year relapse risk prediction nomogram for primary AIHA. The nomogram has a *C*-index of 0.852 (95% CI: 0.797–0.907), confirmed by bootstrapping validation as 0.829. The area under the ROC was 0.846. The DCA shows that when the threshold probability is in the range of 1 ~ 91%.

Conclusions: By following the current diagnostic and treatment criteria for AIHA in China, we retrospectively collect a multitude of medical records and analyze several relevant variables of AIHA, construct a predictive model by machine learning. Using this 1-year relapse risk nomogram can effectively predict the risk of relapse within 1 year after remission of primary AIHA.

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Autoimmune haemolytic anemia; relapse; machine learning; nomograms

Introduction

Autoimmune Haemolytic Anaemia (AIHA) is defined as the increased destruction of red cells through autoimmune mechanisms, usually mediated by autoantibodies against erythrocyte surface antigens [1,2]. The incidence of AIHA is considered uncommon, with prior estimates of 1 to 3 in 100,000 population annually [3]. According to the optimal reaction temperature between autoantibodies and red blood cells, AIHA can be divided into warm antibody type (wAIHA, accounting for 60%~80%), cold antibody type (cAIHA, accounting for 20%~30%), and mixed type (accounting for about 5%) [4]. It can arise either because of primary tolerance breakage or along with several associated conditions, including genetic predispositions, congenital syndromes, environmental triggers, autoimmune diseases, immunodeficiencies, and neoplasms [5]. Unlike secondary AIHA induced or promoted by various diseases or medications, primary AIHA exhibits heterogeneous development patterns among individuals. The treatment of AIHA typically involves glucocorticoids, rituximab, immunosuppressants, and blood transfusion. The therapeutic approach varies depending on the specific types of AIHA. Additionally, several novel drugs targeting its underlying pathogenesis are still under development. Given that this disease can be

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life-threatening for some individuals, transfusions in AIHA are typically reserved for severe cases or administered before a definitive diagnosis is confirmed. However, the temporal sequence of second-line options is still controversial and different in different countries, and only a few predictors of outcome are available [6].

During the last decades, several new established or investigational therapies have appeared, resulting in improvements of therapy but also raising medical and financial challenges on how to treat individual patients. Incomplete diagnostic workup, overuse of corticosteroids, lack of access to more specific treatments, and poor follow-up of patients are the rule more than exceptions in some low-to-middle income countries [7]. Considering the complexity of the disease group and the necessity for an effective evaluation of disease management at the individual level, the development of a relapse prediction model is necessary. It is crucial to incorporate not only clinical characteristics but also biomarkers that are associated with pathophysiological differences and severity to enhance the accuracy of prediction models and facilitate the selection of the optimal therapeutic approach [8]. Machine learning as an artificial intelligence branch that includes computers to learn progressively from examples, data, and experience. It is a technique for teaching computers how to manage data more effectively [9]. Current machine learning advancements in healthcare have primarily served as a supportive role in a physician or analyst's ability to fulfil their roles, identify healthcare trends, and develop disease prediction models [10]. The objective of this study is to develop a machine learning-based relapse prediction model for primary AIHA. The model was constructed using training and test sets derived from clinical data obtained from previous cases of primary AIHA, along with their 1-year follow-up outcomes (relapse/non-relapse). The establishment of this predictive model aims to facilitate: (1) early prediction for patients at high risk of relapse to improve diagnosis and treatment planning in primary AIHA; (2) exploration of potential risk factors contributing to the relapse of primary AIHA; (3) development of more precise follow-up plans and outpatient treatment strategies.

Methods

Patients' information

Our analysis focused on cases of primary AIHA that were treated at the Affiliated Hospital of Southwest Medical University and Xuyong County People's Hospital between May 2017 and May 2022. We searched the hospital's information system (HIS) and laboratory information system (LIS) to identify cases that had a record of hospitalization, as well as a referral outcome (remission or not) within 1 year. When the same patient was admitted to the hospital multiple times within 5 years, we allow multiple inclusions based on the medical records. Patients with haematopoietic and lymphoproliferative diseases, Castleman's disease, myelofibrosis, solid tumours were excluded. Additionally, a follow-up for 1 year was performed on patients who achieved remission (electronic case retrieval and telephone inquiries are used in combination). The study observed and recorded the demographic data, diagnostic information, treatment protocols, and comorbidities of these patients throughout their hospitalization and follow-up period. The study has received ethical approval from the Ethics Committee of the Affiliated Hospital of Southwest Medical University and the patient has provided verbal consent for telephone inquiries.

Inclusion criteria and relapse assessment for primary AIHA cases

Inclusion criteria included

(1) A definitive diagnosis of primary AIHA, including (A) Haemoglobin levels meeting criteria for anaemia, (B) Detection of red blood cell (RBC) autoantibodies, and (C) Compliance with at least one of the following: reticulocyte percentage >4% or absolute value $>120\times10^9$/L; haptoglobin <100 mg/L; total bilirubin ≥17.1 μmol/L (predominantly elevated unconjugated bilirubin). As well as (2) Achieved remission post-treatment, which was necessary to fulfil one of the following criteria: (A) Achievement of complete remission (CR), characterized by the disappearance of clinical symptoms, restoration of normal RBC level, and reticulocyte percentage, serum bilirubin levels. Both the direct antiglobulin test (DAT) and indirect antiglobulin test (IAT) should yield negative results. (B) Partial remission (PR), clinical symptoms basically disappeared, haemoglobin > 80 g/L, reticulocytes percentage <4%, serum total bilirubin < 34.2 μmol/L. DAT may either be negative or still positive but with significantly reduced titre.

Relapse criteria

(1) Achieved the following laboratory indicators, including (A) Haemoglobin < 80 g/L reappeared within 1 year after remission (including CR and PR); (B) Autoantibodies exhibiting a higher agglutination

intensity compared to that observed during the previous remission were detected; (C) any of the following: reticulocytes percentage >4% or absolute value $>120\times10^9$/L, haptoglobin < 100 mg/L, or total bilirubin ≥17.1 μmol/L; or (2) developed new serious complications, such as severe infection, thromboembolism, acute renal failure; or (3) death.

Due to the inherent heterogeneity of primary AIHA, achieving a consensus on the factors influencing disease relapse remains elusive within the current discourse. Accordingly, based on prior research, the predictors to be incorporated in this investigation encompass gender; age; initial haemoglobin levels upon admission (Hb, the haemoglobin grouping is categorized based on the transfusion threshold as outlined in the 2022 AIHA treatment guidelines in China); total bilirubin (TBIL, the subgroup is divided into less than twice, four times, and more than four times the basic threshold); reticulocyte rate (The group is categorized into less than five times, 10 times, or more than 10 times the basic threshold); DAT results (IgG+; C3d+; mixed positive); strength of agglutination in DAT; indirect antiglobulin test (IAT) results; total RBC units transfused during hospitalization; the management of intravenous methylprednisolone, intravenous immunoglobulins (IVIG), recombinant human erythropoietin, venous thromboembolism (VTE) prophylaxis, plasma exchange, glucocorticoids, and immunosuppressants; rituximab therapy; splenectomy; multiline therapy (receiving two or more treatment regimens); multiple treatments (being admitted to the hospital more than twice); complicating immune thrombocytopenia (ITP); and complicating infections. The original data is recorded in Supplementary Table.

Data statistics and machine learning modelling

All data, including demographic, diagnosis, treatment, and follow-up characteristics, were expressed as count (%). Statistical analysis was performed using the IBM SPSS Statistic 26 and R software (Version 4.2.2; https://www.R-project.org).

Differences between cases with relapse and non-relapse were tested using either a Mann-Whitney U test, t-test, or χ^2 test as appropriate [11,12]. The least absolute shrinkage and selection operator (LASSO) method, which is suitable for the reduction in high dimensional data because of its good accuracy and its linear nature that causes model complexity to be simpler than non-linear feature selection [13], was used to select the optimal predictive features in risk factors from the cases with primary AIHA. Features with nonzero coefficients in the LASSO regression model were selected [14]. Then, multivariable logistic regression analysis was used to build a predicting model by incorporating the feature selected in the LASSO regression model [15]. The features were considered as odds ratio (OR) having a 95% confidence interval (CI) on both sides and as a *p*-value. All variables with a *p*-value < 0.05 were included as potential predictors in the following predicting model construction for the 1-year relapse of primary AIHA.

Calibration curves were plotted to evaluate the calibration of the primary AIHA nomogram for 1-year relapse. A significant test statistic indicates imperfect calibration [15]. To quantify the effectiveness of the primary AIHA nomogram in predicting 1-year relapse, we measured the discrimination performance using Harrell's *C*-index. We conducted a bootstrapping validation with 1,000 bootstrap resamples to check for overfitting and calculated a relatively corrected *C*-index. To ensure the accuracy of the nomogram, we drew a receiver-operating curve (ROC) and calculated the area under the curve (AUC) [16]. To assess the discriminatory performance of the 1-year relapse of the primary AIHA nomogram, Decision Curve Analysis (DCA) was employed to evaluate the clinical utility by quantifying the net benefits at various threshold probabilities within the primary AIHA cohort [17]. The net benefit was determined by subtracting the percentage of patients who received false positive results from the percentage of patients who truly tested positive. This calculation also takes into account the potential harm of not intervening versus the negative effects of an unnecessary intervention [18].

Results

Patient data

The research flow chart is depicted in Figure 1. A total of 232 cases with primary AIHA were enrolled in the Affiliated Hospital of Southwest Medical University and Xuyong County People's Hospital from May 2017 to May 2022. The cohort consisted of 61 males and 171 females, aged between 15 to 86 years, with a mean age of 52.09 ± 16.22 years. The initial haemoglobin levels of all patients upon admission ranged from 24 to 91 g/L, with a mean value of 56.88 ± 15.61 g/L. The percentage of reticulocytes ranged from 0.40 to 56.10%, with a median value of 12.42%. The total bilirubin levels ranged from 3.9 to 165.3 μmol/L, with a median value of 43.70 μmol/L. The total units of RBC transfusions administered during hospitalization varied from 0 to 13.5 u, with a median value of 2.0 u. The patients

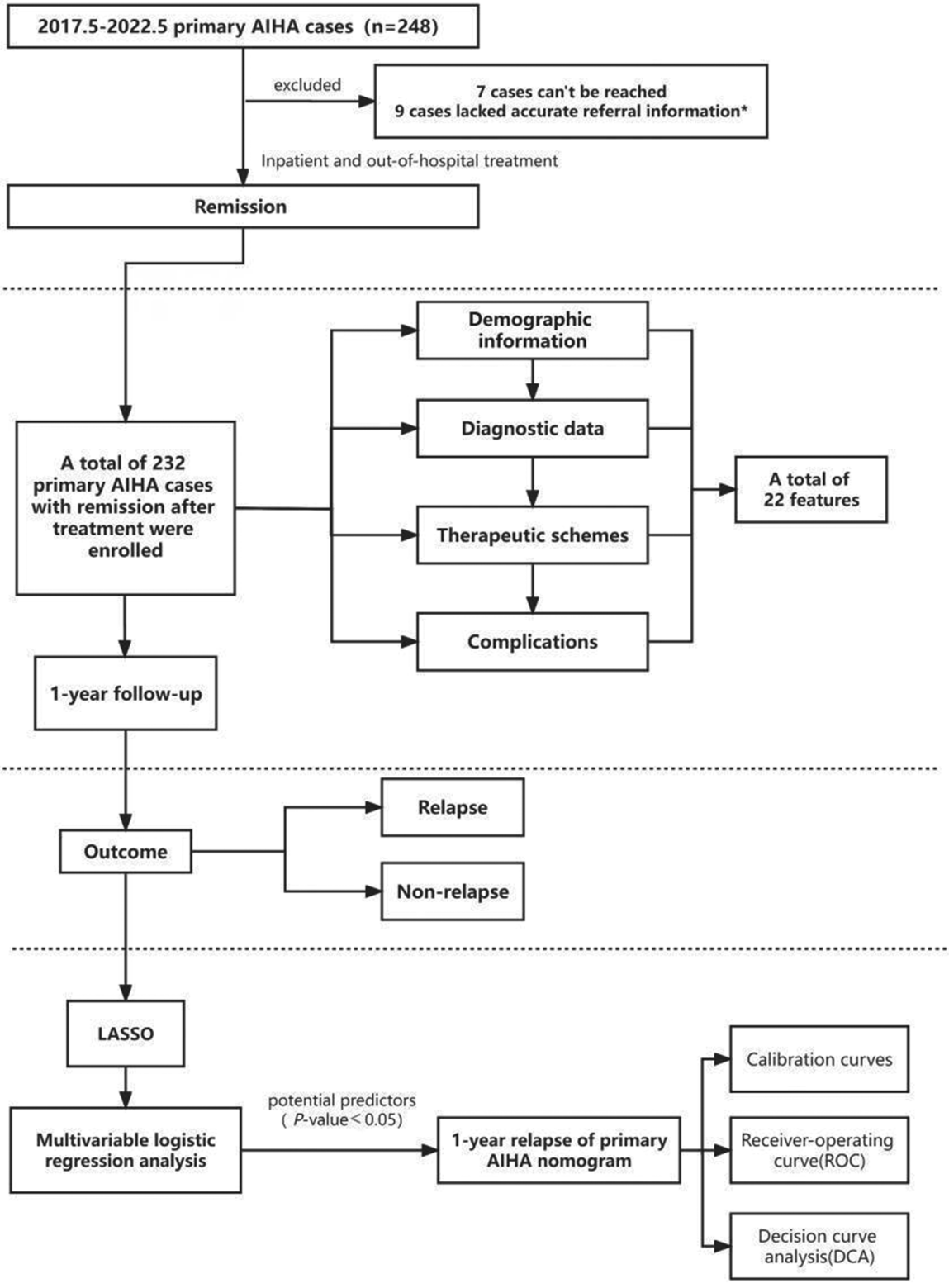

Figure 1. The research flowchart.
Notes: Patients who do not achieve a CR or PR standard in referral within 1 year after discharge are classified as relapse cases. Patients who do not have any referral within 1 year after discharge and are not readmitted due to related conditions are defined as non-relapse cases. *Patients with unknown outcomes (remission or not) of the referral done by another medical institution within 1 year after discharge are excluded from the study.
Abbreviations: CR: Complete Remission; PR: Partial Remission.

were categorized into relapsed cases and non-relapsed cases based on the 1-year follow-up after achieving remission. Among them, there were 74 relapse cases (31.9%) and 158 non-relapse cases (68.1%). The remaining demographic and clinical data are presented in Table 1.

Table 1. Differences in demographic and clinical characteristics between the relapse and non-relapse groups.

Characteristics	*n* (%) 1-year relapse (*n* = 74)	Non-relapse (*n* = 158)	Total (*n* = 232)	*p*-value (1-year relapse vs. non-relapse)
Gender				0.714
Male	18 (24.3)	43 (27.2)	61 (26.3)	
Female	56 (75.7)	115 (72.8)	171 (73.7)	
Age				0.516
<30	6 (8.1)	14 (8.9)	20 (8.6)	
30 ~ 59	44 (59.5)	94 (59.5)	138 (59.5)	
≥60	24 (32.4)	50 (31.6)	74 (31.9)	
DAT results				<0.001
IgG+	5 (6.8)	61 (38.6)	66 (28.4)	
C3d+	9 (12.2)	24 (15.2)	33 (14.2)	
Mixed positive	60 (81.1)	73 (46.2)	133 (57.3)	
Strength of agglutination in DAT				0.002
1+	1 (1.4)	18 (11.4)	19 (8.2)	
2+	1 (1.4)	16 (10.1)	17 (7.3)	
3+	25 (33.8)	50 (31.6)	75 (32.3)	
4+	47 (63.5)	74 (46.8)	121 (52.2)	
IAT results				0.213
Negative	26 (35.1)	67 (42.4)	93 (40.1)	
Positive	48 (64.9)	91 (57.6)	139 (59.9)	
Hb				<0.001
<50 g/L	34 (45.9)	31 (19.6)	65 (28.0)	
50 ~ 69 g/L	32 (43.2)	80 (50.6)	112 (48.3)	
≥70 g/L	8 (10.8)	47 (29.7)	55 (23.7)	
Percentage of reticulocyte				0.173
<4%	4 (5.4)	19 (12.0)	23 (9.9)	
4 ~ 19.99%	51 (68.9)	102 (64.6)	153 (65.9)	
20 ~ 39.99%	19 (25.7)	35 (22.2)	54 (23.3)	
≥40%	0 (0.0)	2 (1.3)	2 (0.9)	
TBIL				0.068
<17.1 μmol/L	9 (12.2)	31 (19.6)	40 (17.2)	
17.1 ~ 34.1 μmol/L	18 (24.3)	37 (23.4)	55 (23.7)	
34.2 ~ 68.3 μmol/L	28 (37.8)	61 (38.6)	89 (38.4)	
≥68.4 μmol/L	19 (25.7)	29 (18.4)	48 (20.7)	
Units of blood transfusion				0.064
<2 u	28 (37.8)	73 (46.2)	101 (43.5)	
2 ~ 7.5 u	39 (52.7)	76 (48.1)	115 (49.6)	
≥8 u	7 (9.5)	9 (5.7)	16 (6.9)	
Intravenous methylprednisolone				0.147
No	10 (13.5)	34 (21.5)	44 (19.0)	
Yes	64 (86.5)	124 (78.5)	188 (81.0)	
IVIG				0.357
No	50 (67.6)	116 (73.4)	166 (71.6)	
Yes	24 (32.4)	42 (26.6)	66 (28.4)	
Recombinant human erythropoietin				0.417
No	73 (98.6)	151 (95.6)	224 (96.6)	
Yes	1 (1.4)	7 (4.4)	8 (3.4)	
VTE prophylaxis				0.031
No	71 (95.9)	137 (86.7)	208 (89.7)	
Yes	3 (4.1)	21 (13.3)	24 (10.3)	
Plasma exchange				1.000
No	73 (98.6)	155 (98.1)	228 (98.3)	
Yes	1 (1.4)	3 (1.9)	4 (1.7)	
Glucocorticoid treatment				0.829
No	5 (6.8)	8 (5.1)	13 (5.6)	
Yes	69 (93.2)	150 (94.9)	219 (94.4)	
Immunosuppressant therapy				<0.001
No	61 (82.4)	90 (57.0)	151 (65.1)	
Yes	13 (17.6)	68 (43.0)	81 (34.9)	
Rituximab				0.713
No	73 (98.6)	153 (96.8)	226 (97.4)	
Yes	1 (1.4)	5 (3.2)	6 (2.6)	
Splenectomy				1.000
No	73 (98.6)	155 (98.1)	228 (98.3)	
Yes	1 (1.4)	3 (1.9)	4 (1.7)	
Multiline therapy				<0.001
No	61 (82.4)	84 (53.2)	145 (62.5)	
Yes	13 (17.6)	74 (46.8)	87 (37.5)	
Multiple treatment				0.688
No	47 (63.5)	96 (60.8)	143 (61.6)	
Yes	27 (36.5)	62 (39.2)	89 (38.4)	

(*Continued*)

Table 1. Continued.

Characteristics	*n* (%) 1-year relapse (*n* = 74)	Non-relapse (*n* = 158)	Total (*n* = 232)	*p*-value (1-year relapse vs. non-relapse)
Complicating ITP				0.001
No	48 (64.9)	136 (86.1)	184 (79.3)	
Yes	26 (35.1)	22 (13.9)	48 (20.7)	
Complicating infection				<0.001
No	17 (23.0)	83 (52.5)	100 (43.1)	
Yes	57 (77.0)	75 (47.5)	132 (56.9)	

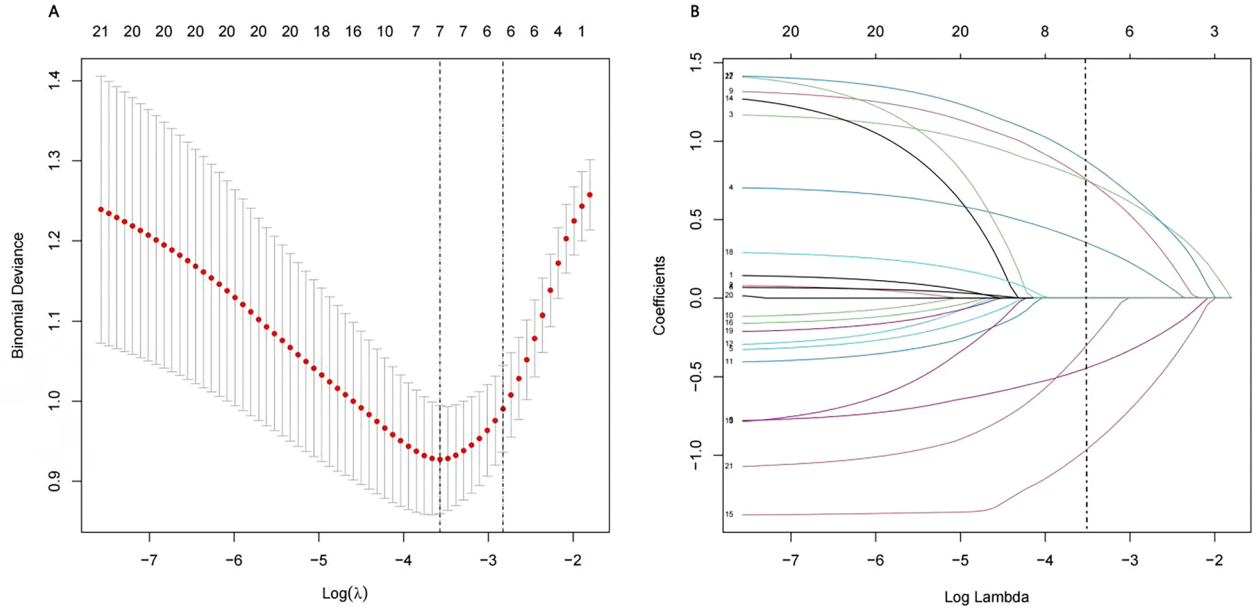

Figure 2. Demographic and clinical feature selection using the LASSO logistic regression model.
Notes: (a) The LASSO model's optimal parameter (lambda) selection used the minimum criteria with fivefold cross-validation. The binomial deviance curve for the partial likelihood deviance was plotted against log(lambda). The optimal values were determined by using the minimum criteria and the 1-SE criteria (the 1 Standard Error criteria), which were marked by dotted vertical lines. (b) The LASSO coefficient profiles of the 22 features were plotted against the log(lambda) sequence. The coefficient profile plot shows that when the optimal lambda was selected using fivefold cross-validation, seven features had nonzero coefficients. A vertical line was drawn at this value to indicate the optimal lambda.
Abbreviations: LASSO: least absolute shrinkage and selection operator; SE: standard error.

Table 2. Potential predictors of 1-year relapse in primary AIHA.

Intercept and valuables	regression coefficient	odds ratio(95% CI)	*p*-value
intercept	−3.9326	0.020 (0.001–0.184)	0.003
DAT results(Mixed positive)	2.3812	10.818 (3.875–37.376)	<0.001(3.53e-05)
Strength of agglutination in DAT (4+)	1.8463	6.337 (1.000–125.735)	0.100
Hb (≥70 g/L)	−1.3045	0.271 (0.086–0.791)	0.020
Complicating ITP (Positive)	1.2836	3.610 (1.431–9.547)	0.008
Multiline therapy (Yes)	−1.5992	0.202 (0.084–0.452)	<0.001(1.7e-04)
Complicating infection (Yes)	1.3587	3.891 (1.824–8.653)	<0.001(5.8e-04)
VTE prophylaxis (Yes)	−1.1349	0.321 (0.060–1.341)	0.143

Optimal feature screening

The demographic and clinical features were initially reduced from 22 to 7 potential predictors based on a cohort of 232 patients (Figure 2a and b), exhibiting nonzero coefficients in the LASSO regression model. These features encompassed 'DAT results', 'Strength of agglutination in DAT', 'Hb', 'Complicating ITP', 'Multiline therapy', 'Complicating infection', and 'VTE prophylaxis.' The aforementioned features were included as covariates in the multivariable logistic regression analysis. The coefficients, odds ratio (OR), and *p*-value are presented in Table 2.

Nomogram

The potential predictors for constructing the prediction model were determined based on variables with a *p*-value < 0.05. Therefore, the variables 'Strength of agglutination in DAT' and 'VTE prophylaxis' were excluded

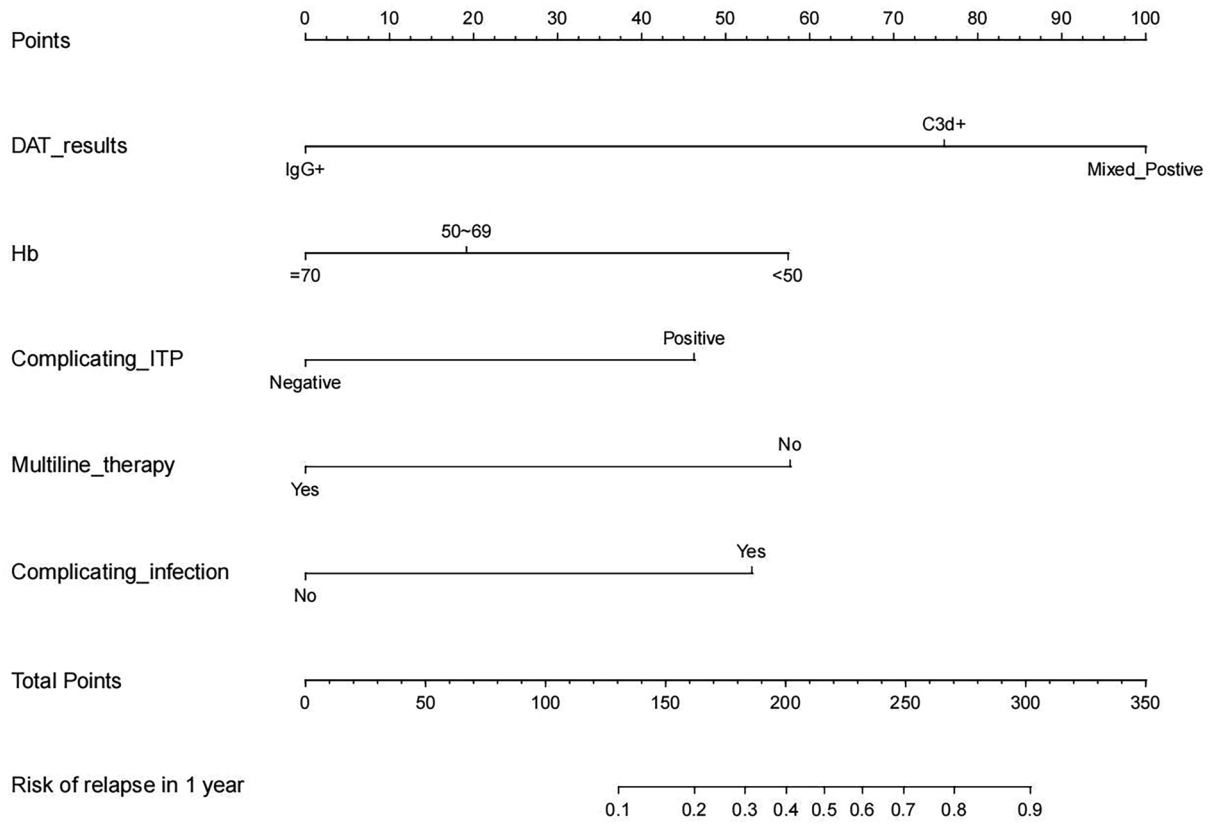

Figure 3. Development of a prognostic nomogram for predicting 1-year relapse risk in patients with primary AIHA.
Note: The 1-year relapse nomogram was developed in the cohort, with the 'DAT results', 'Hb', 'Complicating ITP', 'Multiline therapy', and 'Complicating infection'.
Abbreviations: AIHA: autoimmune haemolytic anaemia.

from consideration. The variables 'DAT results', 'Hb', 'Complicating ITP', 'Multiline therapy', and 'Complicating infection' were included in the development of the model and presented as a nomogram (Figure 3).

Apparent performance of the 1-year relapse risk nomogram in the cohort

The calibration curve of the nomogram for predicting 1-year relapse risk in primary AIHA patients exhibited excellent concordance within this cohort (Figure 4). The *C*-index for the prediction nomogram was 0.852 (95% CI: 0.797–0.907) for the cohort and was confirmed to be 0.829 through bootstrapping validation, which suggested the model's good discrimination and demonstrated a strong predictive capability. The receiver-operating curve (ROC) was plotted, and the area under the curve (AUC) was determined to be 0.846, indicating a high level of accuracy for the 1-year relapse risk nomogram (Figure 5).

Clinical use

The decision curve analysis for the 1-year relapse risk nomogram is presented in Figure 6. The decision curve

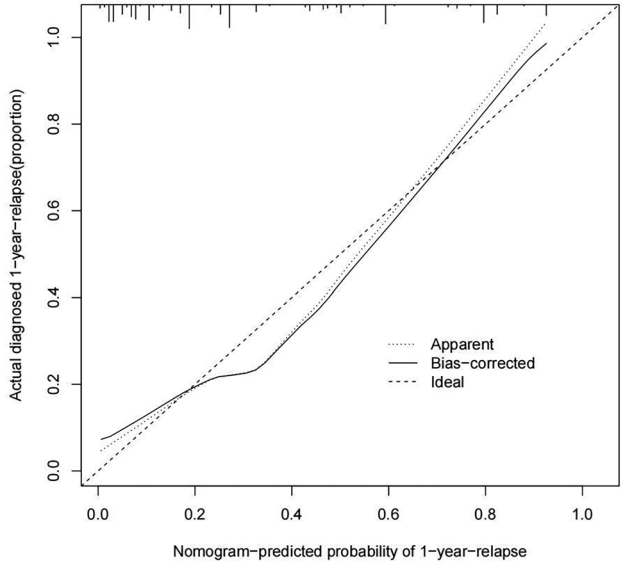

Figure 4. Calibration curves of the 1-year relapse nomogram prediction in the cohort.
Notes: On the graph, the x-axis is used to represent the predicted 1-year relapse risk, while the y-axis represents the actual diagnosed relapse in 1 year after remission. The ideal model is represented by the dotted diagonal line, while the nomogram's performance is represented by the solid line. The closer the solid line is to the dotted diagonal line, the better the prediction.

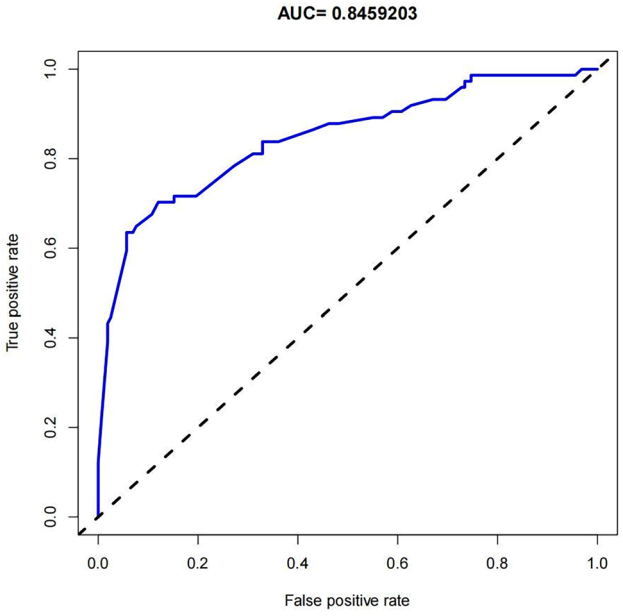

Figure 5. ROC and AUC of the 1-year relapse nomogram prediction in the cohort.
Notes: The ROC depicts the relationship between sensitivity and specificity of the 1-year relapse nomogram model. The x-axis represents the false positive rate, with a closer proximity to zero indicating higher accuracy, i.e. lower probability of incorrect relapse prediction by the model. The y-axis represents the true positive rate. A larger value on the y-axis signifies higher accuracy, i.e. a greater likelihood of correct relapse prediction by the model. Based on the position of the blue curve, two distinct regions can be identified in this graph. The area under the blue curve is referred to as AUC, which serves as an indicator for prediction accuracy. A larger AUC corresponds to a more accurate prediction model, while a closer alignment of the curve towards the upper left corner indicates superior performance.
Abbreviations: ROC: receiver operator characteristic curve; AUC: the area under the curve

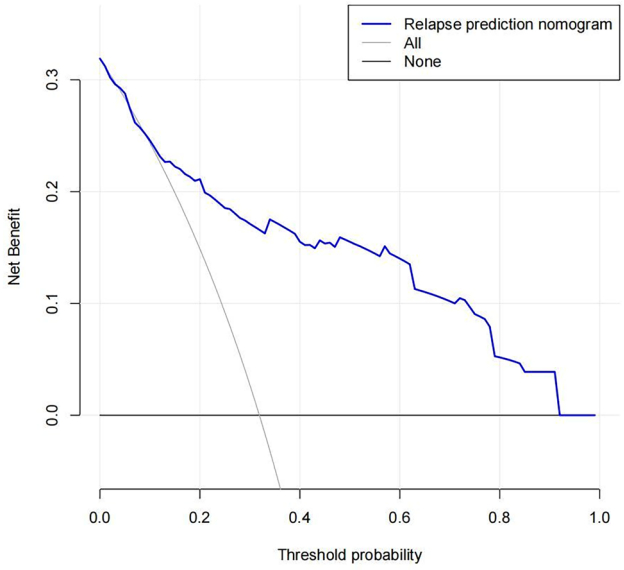

Figure 6. Decision curve analysis for the 1-year relapse nomogram.
Notes: The graph has the threshold probability on the x-axis and the net benefit on the y-axis. The blue line shows the 1-year relapse risk nomogram. The thin solid line assumes that all patients will relapse within a year, while the thick solid line assumes that no patient will. The decision curve indicates that if a patient's threshold probability is between 1% and 91%, using this nomogram to predict the risk of relapse within a year will be more beneficial than either intervening with all patients or not intervening at all.

showed that if the threshold probability of a cohort falls within the range of 1 ~ 91%, using this 1-year relapse risk nomogram to predict 1-year relapse risk adds more benefit than the normal scheme.

Discussion

The lack of effective prognostic evaluation and prediction methods for primary AIHA poses challenges in determining the most optimal treatment and prevention strategies, thereby impeding patients and physicians from making informed decisions. To investigate the risk factors associated with relapse in primary AIHA and facilitate early identification of high-risk patients, as well as develop more precise diagnostic and treatment strategies for individuals with primary AIHA, we developed a machine learning-based nomogram model to predict the 1-year relapse risk following remission. The prediction model included a total of five features following dimensionality reduction using the LASSO regression model, including DAT results, haemoglobin value at admission, complicating ITP, multiline therapy, and complicating infection. Among them, the presence of C3d + or mixed positive results in DAT, concurrent ITP, and complicating infection are identified as risk factors for inducing relapse of primary AIHA. Conversely, the onset of haemoglobin levels exceeding 70 g/L and the utilization of multiline therapy may serve as protective factors against AIHA relapse. The 1-year relapse nomogram presented herein serves as a valuable tool for physicians in the timely identification of high-risk patients susceptible to haemolysis relapse, thereby facilitating prompt adjustment of treatment plans.

The features included in the analysis were primarily obtained from demographic, diagnostic, treatment, and comorbidity data to effectively capture the heterogeneity observed among cases, particularly with regard to treatment regimens. Current treatments for primary AIHA include emergency therapy(including RBC transfusions, Intravenous methylprednisolone, plasma exchange, etc.), general measures (including thromboembolism prophylaxis and/or recombinant human erythropoietin use), and specific therapies (depend on warm AIHA and AIHA from CAD) [19]. In our study, for the efficient and concise construction of the predictive model, specific treatment regimens were categorized into first-line steroid therapy and

second-line therapies, including cytotoxic immunosuppressants, rituximab, and splenectomy. Sutimlimab-jome as a breakthrough for CAD, approved by FDA in February 2022 as the first therapy specifically for CAD, targeting the classical complement pathway to reduce haemolysis [20]. While effective, its use requires careful patient selection, vaccination, and monitoring for infections. However, in our study, there is no one treated by this medicine. The existing view of second-line treatment remains controversial. Few therapeutics offer treatment-free durable remission [21]. Hence, the impact of current treatments on patient outcomes remains uncertain. In addition, blood transfusion as an emergency treatment is generally reserved for severe or refractory cases. It should be performed on the basis of immunosuppressive therapy (such as glucocorticoids) and minimized due to the risk of alloimmunization [22]. Patients with repeated transfusion may be at risk of inducing AIHA [23]. Therefore, relevant indicators pertaining to blood transfusion therapy were incorporated into the assessment.

The severity of a patient's anaemia at the time of onset is important for predicting relapse and mortality in primary AIHA, according to a large multicentre study led by Dr. Barcellini [6]. In this study, Multivariate Cox regression analysis demonstrated that anaemia severity at onset was associated with an increased risk of relapse, with the following hazard ratios: 1.98 (95% CI 1.22–3.21) for patients with Hb ≤ 6 g/dL, 1.74 (95% CI 1.09–2.77) for cases with Hb 6.1–8 g/dL, and 1.61 (95% CI 0.99–2.62) for those with Hb 8.1–10 g/dL. Even considering haemoglobin as a continuous variable, each gram of reduction yielded a 7% greater risk of relapse (95% CI 2–13, $p < .013$). This is consistent with the results of our study, which found that compared to Hb < 50 g/L, the cases with Hb in 50 ~ 69 g/L or Hb ≥ 70 g/L at the onset, the OR values of relapse were 0.410 (95% CI 0.17–0.94) and 0.271 (95% CI 0.08–0.79), respectively. These results indicate a higher 1-year relapse risk associated with Hb levels at disease onset. Furthermore, previous studies have shown a higher risk of severe clinical course and mortality for complement-positive AIHA, mixed and atypical AIHA (IgM warm AIHA) [24]. This finding aligns with the outcome of our study, indicating that individuals with C3d + or mixed positive DAT results have a higher likelihood of relapse within 1 year following remission. According to the existing studies, this may be related to the opsonization or intravascular haemolysis caused by complement activation [22]. More tests may be needed to confirm this theory. Previous tests have demonstrated that infections, particularly following splenectomy, acute renal failure, and Evans syndrome, serve as prognostic indicators for fatal outcomes [25]. Through multivariable logistic regression analysis, this study has identified haemolysis accompanied by ITP as a significant risk factor for patient relapse. The findings also provide further evidence that both in-hospital and out-of-hospital infections, such as pulmonary infection, urinary tract infection, tuberculosis, etc., may contribute to an increased risk of relapse in primary AIHA. This suggests that stringent infection control measures could serve as a crucial approach to facilitate sustained remission. Regarding the impact of therapeutic schedules on prognosis, these findings do not entirely align with previous research outcomes. The results of a meta-analysis indicate that the combination therapy involving rituximab and glucocorticoid may significantly enhance the rate of complete hematological response within 12 months compared to glucocorticoid monotherapy [26]. However, Barcellini proved that multi-treatment was one of the predictors of fatal outcomes [25]. According to our nomogram model, multiline therapy demonstrates efficacy in reducing the 1-year relapse rate across the entire patient population. The observed discrepancy in outcomes may be attributed to the definition of multi-treatment provided by Barcellini et al. which specifically refers to the utilization of four or more treatment plans. This particular approach is typically suitable for patients with relapsed/refractory (R/R) disease or severe cases. In contrast, multiline therapy in our study encompasses the use of two or more treatment regimens. A total of 78 cases (89.7%) received a combination therapy of steroids and cyclosporine, while 5 cases (5.7%) were treated with steroids and rituximab; in addition, splenectomy supplement to steroid was performed in 3 cases (3.4%). One case (1.1%) received a multiline therapy consisting of steroids, cyclosporine and splenectomy. According to the DCA curve, this 1-year relapse nomogram has the potential to serve as a valuable intervention tool within a threshold probability range of 1% to 91%. Early implementation of multiline therapy for patients identified as high-risk can effectively delay the recurrence of haemolytic anaemia.

Previous studies have shown mixed AIHA [defined by the presence of warm IgG autoantibodies and cold agglutinins with a high-thermal-amplitude (>30 °C)] accounts for about 6 ~ 8% in whole cases [27], which differs from the definition employed in our study, where 'mixed positive' DAT results were defined as being positive for both anti-IgG and anti-C3d. Consequently, the prevalence of cases exhibiting mixed positive DAT results is remarkably high at 57.3%. Additionally, our findings indicate that within different disease conditions, a single patient may exhibit

varying DAT results over time; for instance, initial diagnoses of IgG + can transition to IgG + and C3d + following multiple treatments and vice versa. Currently, the underlying cause of this phenomenon remains unclear, necessitating further investigation into its potential correlation with disease severity, diagnostic techniques, and treatment interventions such as blood transfusion. High titres of antibodies may increase the relapse rate of primary AIHA patients [28]. In a prospective concurrent controlled study, Liu et al. followed 52 AIHA/Evans patients who achieved complete remission after treatment for a period of 1 to 14 years. They found the relapse rate in patients with antibody titre > or = 100 was 92.9% and was higher than that in patients with antibody titre < 100 (59.5%) ($p < 0.05$). Our findings demonstrated that the odds ratios (OR) for 2+, 3+, and 4+ levels of 'Strength of agglutination in DAT' were calculated as 0.635 (95% CI 0.02–20.32), 4.370 (95% CI 0.66–88.32), and 6.337 (95% CI 1.00–125.74), respectively, when compared to cases with 1+. However, due to the fact that the corresponding *p*-values were ≥0.05, they were excluded as predictive features in the nomogram model for 1-year relapse. The inconsistency observed in the results of previous studies was discussed, which could potentially be attributed to the limited duration of follow-up in this study or the categorization of antibody titres based on agglutination strength in DAT. In addition, research shows that transfusions emerged as an independent factor associated with thrombosis (HR 3.06) [29], and some authors concluded that AIHA is a potential complication of allogeneic RBC transfusions [30], that may indicate transfusion will lead to a higher risk of relapse of AIHA. However, our results did not show that the amount of RBC transfused during hospitalization was associated with the outcome of AIHA. The results may vary due to patient heterogeneity between studies and the diverse perspectives of physicians on transfusion, which subsequently influence the final blood volume of patients transfused. To further elucidate this matter, it might be necessary to screen patients as homogeneously as possible and group them accordingly for strict comparison of transfusion thresholds and dosages.

Nowadays, the prediction model provides a new method for clinical diagnosis and treatment of diseases [31–33]. The reasonable prevention and control of primary AIHA, along with the development of a relapse prediction model, holds immense significance in addressing this life-threatening disease. Currently, this field remains unexplored. Based on the research findings, we propose several hypotheses. Firstly, modulation of complement activation (including interventions such as clearance of cold antibody, plasma exchange, immunosuppression, and complement C1 inhibition) may contribute to improving prognosis in cases of primary AIHA with C3d + in DAT. Secondly, efforts should be made to prevent infection and promptly treat any infections in order to minimize the risk of haemolysis relapse. Additionally, employing multiline treatment regimens that patients can tolerate or that have minimal side effects may potentially enhance patient prognosis.

There are still several limitations in this study. Firstly, the limited prevalence of primary AIHA hinders the feasibility of conducting large-scale follow-up studies. Therefore, although aware of the differences in pathophysiology and management between warm and cold AIHA, we are now unable to use these limited cases to construct predictive models for primary wAIHA or cAIHA separately. Secondly, the treatment modality for patients is concurrently influenced by decisions, with the majority of multiline treatments being limited to steroid-cytotoxic drug combinations. In cases of cAIHA, glucocorticoids remain the predominant treatment option, while rituximab is sparingly utilized; thereby, these treatment modalities cannot reflect the impact of the latest treatment mode on the prognosis and relapse of patients. If a transregional multi-center cohort study and external validation of the model can be conducted, the results will be more convincing and clinically significant. Thirdly, the retrospective cohort study used in this study was followed up for only 1 year, so the results of the follow-up may have certain limitations and biases. In addition, some diagnostic and therapeutic indicators that may affect patient outcomes have not been included in studies due to low clinical attention or difficulty in monitoring, such as binding globin and recently proposed Bone Marrow Evaluation [4]. The medication adherence of patients may also affect the relapse of patients out of the hospital, which has a certain value of discussion and deserves further study.

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Ethical approval

This study was approved by the ethics committee of the Affiliated Hospital of Southwest Medical University (approval

no. KY2025036). We certify that the study was performed in accordance with the 1964 declaration of HELSINKI and later amendments.

Verbal consent statement

This study does not involve the disclosure of patients' personal information, nor will it cause any harm to patients' interests. For the patients are often unable to meet directly in the later follow-up process, therefore verbal informed consent was obtained and recorded by the Mobile CallRecorder from all the participants prior to the publication of this study. And this was approved by the ethics committee of the Affiliated Hospital of Southwest Medical University (approval no. KY2025036).

Authors contributions

CRediT: **Pan Li**: Data curation, Formal analysis, Investigation, Writing – original draft; **Chuanqi Zhong**: Data curation, Investigation, Software, Validation, Writing – original draft; **Xianjun Huang**: Data curation, Investigation, Software, Writing – review & editing; **Zhi Cai**: Data curation, Software, Writing – review & editing; **Tianhong Guo**: Data curation, Funding acquisition, Investigation, Project administration, Software, Supervision, Validation, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated or analyzed during this study are included in this publish article and its supplementary information files.

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