Evaluating the added predictive ability of MMP-9 in serum for Kawasaki disease with coronary artery lesions

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ABSTRACT

To investigate the predictive ability of serum matrix metalloproteinase-9 (MMP-9) in the acute phase of Kawasaki disease (KD) with coronary artery lesions (CALs). Patients with KD hospitalized in Lanzhou University Second Hospital, Northwest China, from November 2015 to January 2018 were retrospectively reviewed, and clinical trial indicators and peripheral blood specimens were collected before intravenous immunoglobulin therapy treatment. The independent risk factors were determined using multivariate regression analysis. The net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to quantitatively evaluate the ability of MMP-9 to improve the efficiency of predicting KD with CALs. The white cell, neutrophil percentage, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were higher in patients with higher MMP-9, and the monocyte percentage was higher in patients with lower MMP-9. Logistic regression analysis revealed that long-term fever; elevated CRP, ESR, platelets (PLT), and MMP-9; and low albumin (ALB) levels were independent predictors of KD with CALs. A predictive model of KD with CALs using fever duration, CRP, ESR, PLT, and ALB showed significantly improved predictive ability when MMP-9 was added to the model (the area under the curve increased by 0.02; no change in sensitivity; specificity increased from 81.48% to 87.04%; NRI value: 13.46%; IDI value: 5.00%, p<0.05). Adding MMP-9 to traditional risk factors may improve prediction of CALs, the overall predictive ability of model 2 was increased by 5%.

Significance of this study

What is already known about this subject?

- Kawasaki disease (KD) is one of the most frequent acquired heart diseases in the world. The most severe complication or sequela is the formation of coronary artery lesions (CALs), which may subsequently result in long-term sequelae, such as stenosis, obstruction and myocardial infarction.
- The remodeling of the extracellular matrix (ECM) is a key histological feature of the vascular lesions of KD because ECM molecules constitute the basement membrane that contributes structural support to the vasculature.
- Matrix metalloproteinases (MMPs) are a family of zinc-calcium-dependent endogenous proteolytic enzymes involved in the degradation of ECM. MMP-9 is most closely related to vascular disease.

INTRODUCTION

Kawasaki disease (KD) is an acute autoimmune systemic vasculitis disease that mainly affects young children and is characterized by fever, bilateral conjunctival inflammation, atypical rash, and so on.[1 2] The most serious cardiovascular complications of KD are the result of coronary artery lesions (CALs) caused by inflammation, including coronary artery dilatations, coronary artery aneurysms and coronary artery fistula formations.[3–5] Although the disease can be controlled, 3%–5% of children develop CALs,[6] which may lead to myocardial ischemia or sudden death in severe cases.[7 8] In addition, the resulting coronary arteritis can increase the risk of long-term cardiovascular complications, such as high blood pressure and greater arterial stiffness.[9 10]

The remodeling of the extracellular matrix (ECM) is a key histological feature of the vascular lesions of KD because ECM molecules constitute the basement membrane that contributes structural support to the vasculature.[11 12] Matrix metalloproteinases (MMPs) are a family of zinc-calcium-dependent endogenous proteolytic enzymes involved in the degradation of ECM.[13] MMP-9, also known as gelatinase B, is an important member of the MMP family and can degrade the subendothelial layer mainly composed of collagen tissue, elastin and proteoglycan, which is most closely related to vascular disease.[14–16] Previous studies have reported increased concentrations of MMP-9 during the acute phase of KD, especially for those with CALs, suggesting the potential role of MMP-9 level in CAL prediction.[17–19] However, no one has quantitatively evaluated the predictive

Significance of this study

What are the new findings?

- In addition to traditional risk factors such as long-term fever, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelets (PLT), and albumin (ALB), MMP-9 is an independent risk factor for KD with CALs.
- A predictive model of KD with CALs using fever duration, CRP, ESR, PLT and ALB showed significantly improved overall predictive ability when MMP-9 was added to the model.
- White cell, neutrophil percentage, CRP, and ESR were higher in patients with higher MMP-9 levels, and the monocyte percentage was higher in patients with lower MMP-9.

How might these results change the focus of research or clinical practice?

- This study provides evidence to better understand MMP-9 in predicting KD with CALs and provides clues for its prevention.

ability of serum MMP-9 for KD with CALs. In recent years, the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) have become increasingly popular in the quantitative evaluation of the improvement in the predictive effectiveness of models with the addition of a new risk factor.[20] The NRI illustrates the percentage of a population that is accurately reclassified, providing clinically meaningful results rather than only statistically significant differences between χ^2 and ΔAUC.[20] IDI is used to assess the overall improvement of the prediction model, it summarizes the extent a new model increases risk in events and decreases risk in non-events.[20] The aims of our study were to (1) validate the predictive ability of MMP-9; and (2) use NRI and IDI to quantify the effect of MMP-9 on CALs to predict KD.

MATERIALS AND METHODS

Patients

We retrospectively collected the medical records of patients diagnosed with KD at Lanzhou University Second Hospital from November 2015 to January 2018. All patients were diagnosed with KD according to the criteria established by the Kawasaki Disease Research Committee. The exclusion criteria were as follows: (1) patients with previous recurrence of KD; (2) children with KD who did not respond to the initial treatment and were hospitalized again; and (3) patients with other vascular inflammatory diseases.

Methods

Venous blood samples were collected from all children with KD before intravenous immunoglobulin (IVIG) therapy and centrifuged at 3000 r/min for 5 minutes at low speed. The serum was separated and stored at −80°C.

The amount of MMP-9 and interleukin-6 (IL-6) in serum samples was measured by an ELISA using the MMP-9 and IL-6 assay kit (Catalog No: E-EL-H1451c, E-EL-H0102c; Elabscience, China), according to the manufacturer's instructions. The resultant color was measured at 450 nm in a microtiter plate spectrophotometer. The concentrations of MMP-9 and IL-6 were determined by interpolation from a standard curve. All assays were performed in duplicate.

All demographic characteristics and the laboratory data prior to the initial use of IVIG were collected. The demographic characteristics included age (month), sex, and fever duration; the laboratory data included white cell, neutrophil percentage (NE%), lymphocyte percentage, platelets (PLT), albumin (ALB), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), red cell, hemoglobin, monocyte percentage (MO%), and eosinophil percentage. All those laboratory variables were routinely obtained in clinical practice.

Definition

Complete Kawasaki disease[21]: At least 5 of the following 6 items; or 4 of 6 items, and echocardiographic findings of coronary aneurysms or coronary dilatation: (1) fever persisting for 5 or more days; (2) bilateral conjunctival congestion; (3) changes in the lips and oral cavity; (4) polymorphous exanthema; (5) changes in the peripheral extremities; and (6) acute non-suppurative cervical lymphadenitis. Incomplete Kawasaki disease (IKD) was defined as having 4 or fewer principal signs, with or without cardiac lesions. CALs: Two-dimensional echocardiography was used to measure the coronary artery, carried out by a technician, which was performed before initial treatment and repeated at the point of 1, 2 and 4 weeks after the initial treatment. The size of the inner diameters of the right main coronary artery, left main coronary artery, left anterior descending artery, and left circumflex artery was measured, and measurement was repeated 2 times for each position, with the mean taken as the ultimate value. The patient's attending doctor and superior doctor jointly interpret the measurement results. When the diagnosis results of the 2 doctors are inconsistent, the results are interpreted by a panel of 4 cardiologists. The coronary artery was considered abnormal if the internal lumen diameter was >2.5 mm in children <3 years of age, >3 mm in children 3–9 years of age, and >3.5 mm in children 9–14 years of age; the internal diameter of a segment was measuring ≥1.5 times that of an adjacent segment; and the lumen was clearly irregular.

Statistics

All analyses were performed using the R statistical software package (R statistics V.3.5.3). Categorical data are expressed as frequencies (percentages) and compared by the χ^2 test. Normally distributed data are expressed as the mean±SD and analyzed by Student's t-test; continuous data with a non-normal distribution are expressed as the median (P_{25}–P_{75}) and analyzed by the rank-sum test. Multivariate logistic regression analysis of the OR and 95% CI was used to determine independent risk factors for CAL. Both models were tested for collinearity, and no significant collinearity was found in all variables. A receiver operating characteristic (ROC) curve and the area under the curve (AUC) were used to determine the predictive ability, sensitivity and specificity of the prediction model. NRI and IDI[20]

Table 1 Univariate analysis comparison of clinical and laboratory indexes in CAL and NCAL

Variable	CAL (n=76)	NCAL (n=54)	P value
Male, n (%)	60 (78.90)	30 (55.60)	0.004
Age (mo)	11.25 (23.00–41.00)	18.25 (28.00–50.00)	0.213
Fever duration (d)	8 (6–13)	7 (5–10)	0.040
IKD, n (%)	47 (61.80)	24 (44.40)	0.050
White cell ($\times10^9$/L)	13.70 (8.30–19.00)	14.43 (9.51–18.52)	0.606
NE%	0.65 (0.37–0.86)	0.64 (0.48–0.87)	0.634
LY%	0.38 (0.18–0.59)	0.35 (0.20–0.56)	0.478
PLT ($\times10^9$/L)	385.00 (280.00–482.00)	295.00 (220.00–364.00)	0.001
ALB (g/L)	31.00 (28.90–33.90)	34.90 (32.80–37.60)	<0.001
CRP (mg/L)	41.00 (11.00–112.84)	19.00 (6.00–53.53)	0.009
ESR (mm/h)	89.92±25.56	62.00±31.00	<0.001
Red cell ($\times10^{12}$/L)	4.27±0.59	4.23±0.46	0.700
HGB (g/L)	110.68±16.64	113.51±9.93	0.232
MO%	0.07 (0.05–0.09)	0.07 (0.05–0.10)	0.659
EO%	0.02 (0.01–0.05)	0.01 (0.01–0.03)	0.508
MMP-9 (ng/mL)	845.66 (331.08–1719.53)	593.39 (227.32–814.61)	0.048
IL-6 (pg/mL)	41.10 (18.94–167.44)	38.64 (14.31–83.27)	0.149

Data are mean±SD, P50 (P25–P75) and n (%).
ALB, albumin; CAL, coronary artery lesion; CRP, C-reactive protein; EO%, eosinophil percentage; ESR, erythrocyte sedimentation rate; HGB, hemoglobin; IKD, incomplete Kawasaki disease; IL-6, interleukin-6; LY%, lymphocyte percentage; MMP-9, matrix metalloproteinase-9; MO%, monocyte percentage; NCAL, non-CAL; NE%, neutrophil percentage; PLT, platelet.

were performed to evaluate whether MMP-9 could improve the efficiency of predicting CALs.

RESULTS

Comparison between the CAL group and the group without CAL by univariate analysis

A total of 130 patients with KD were enrolled in the study. The male to female ratio was 2.25:1, and the median age was 28 months (range: 12–43 months). Fifty-nine of those (45.38%) had IKD, and 76 (58.46%) had coronary involvement.

According to univariate analysis (table 1), in patients with CAL, the levels of PLT count, CRP, ESR and MMP-9 were higher, the levels of ALB were lower, male patients and patients with IKD were more common, and fever duration was longer.

Comparison of the characteristics of the patients with KD according to the level of MMP-9

According to the ROC curve analysis, the optimal cut-off value of MMP-9 level on admission for predicting CALs was 1078.68 ng/mL. To evaluate the relationship between MMP-9 and clinical outcome, patients were divided into 2 groups according to this cut-off value (>1078.68 vs ≤1078.68).

The properties were compared between the 2 groups (table 2). White cell count, NE%, CRP, and ESR were higher in patients with higher MMP-9 levels, and the MO% was lower in patients with higher MMP-9 levels. In addition, patients were younger in the lower MMP-9 group.

Analysis of independent risk factors and establishment of the prediction model

Among the 8 variables, 6 indicators presented statistical significance and were used for multivariate logistic regression analysis. The independent risk factors were longer fever; elevated CRP, ESR, PLT, and MMP-9; and low ALB levels. The OR values (95% CI) of those risk factors are listed in table 3.

Based on the above result, a nomogram was derived for personal risk probability of KD with CAL (figure 1). The goodness of fit of the nomogram was assessed by producing a calibration plot, which revealed good agreement between the predicted and observed probabilities (online supplemental figure 1). The underlying logistic model is given by the following equation:

$$\begin{aligned}\text{Logit}(P = CAL) = {} & 0.173 \times (\text{fever duration}) \\ & +0.006 \times (PLT) - 0.143 \times (ALB) \\ & +0.02 \times (CRP) + 0.025 \times (ESR) \\ & +0.001 \times (MMP - 9) - 0.754\end{aligned}$$

Thus, the individual risk probability of patients with KD with CALs could be identified. The coefficients indicate the contribution of the variables. The increase in fever duration, PLT, CRP, ESR and MMP-9 with positive coefficients increased the OR of CAL; the increase in ALB with negative coefficients decreased the likelihood of CAL.

Effect of adding MMP-9 to coronary lesion risk prediction models

Prediction model 1 (AUC was 0.87, sensitivity was 81.58%, specificity was 81.48%) was constructed based on traditional independent risk factors (fever duration time, PLT, ALB, CRP, ESR), and MMP-9 was added on the basis of traditional risk factors to construct prediction model 2 (AUC was 0.89, sensitivity was 81.53%, specificity was 87.04%). Compared with model 1, model 2 had an increase in area under the ROC curve of 0.02, and p=0.10 showed no significant difference (figure 2).

Table 2 Comparison of the characteristics of the patients with Kawasaki disease according to the level of MMP-9

Variable	MMP-9 >1078.68 (n=37)	MMP-9 ≤1078.68 (n=93)	P value
Male, n (%)	24 (64.90)	66 (71.00)	0.496
Age (mo)	34 (15–50)	27 (12–42)	0.020
Fever duration (d)	7 (6–12)	7 (5–12.5)	0.159
IKD, n (%)	18 (48.60)	53 (57.00)	0.389
White cell ($\times10^9$/L)	15.16 (10.58–19.95)	12.49 (8.50–17.82)	0.031
NE%	0.80 (0.53–1.50)	0.58 (0.40–0.82)	0.019
LY%	0.28 (0.15–0.52)	0.42 (0.29–0.66)	0.143
PLT ($\times10^9$/L)	348.00 (280.00–441.50)	312.00 (223.50–416.00)	0.272
ALB (g/L)	31.40 (29.30–34.75)	33.50 (30.00–36.20)	0.131
CRP (mg/L)	88.00 (18.50–131.00)	21.00 (7.87–53.53)	0.001
ESR (mm/h)	89.57±26.40	73.44±31.54	0.007
Red cell ($\times10^{12}$/L)	4.28±0.59	4.24±0.52	0.656
HGB (g/L)	113.70±15.39	111.14±13.75	0.356
MO%	0.05 (0.03–0.08)	0.07 (0.05–0.10)	0.012
EO%	0.01 (0.00–0.03)	0.02 (0.01–0.05)	0.157
IL-6 (pg/mL)	71.37 (22.66–141.89)	37.71 (14.41–95.64)	0.075
CAL, n (%)	33 (46.24)	43 (89.20)	0.001

Data are mean±SD, P50 (P25–P75) and n (%).
ALB, albumin; CAL, coronary artery lesion; CRP, C-reactive protein; EO%, eosinophil percentage; ESR, erythrocyte sedimentation rate; HGB, hemoglobin; IKD, incomplete Kawasaki disease; IL-6, interleukin-6; LY%, lymphocyte percentage; MMP-9, matrix metalloproteinase-9; MO%, monocyte percentage; NE%, neutrophil percentage; PLT, platelet.

We used the logistic regression model to predict the probability of a CAL event. Reclassification tables for subjects who did not have an event and non-development events were constructed using ~0%, ~20%, ~40%, ~60%, and ~80% CAL risk categories (online supplemental table 1). We found that the addition of MMP-9 to the model 1 actually results in more patients being reclassified. For patients with CALs, 17.11% (13/76) were correctly reclassified as higher risk categories, whereas 9.21% (7/76) were incorrectly reclassified as lower risk categories, resulting in a net of 7.90% of the population being correctly reclassified. Conversely, for patients without CALs, the addition of MMP-9 resulted in 20.37% (11/54) being correctly reclassified and 14.81% (8/54) being incorrectly reclassified, yielding a net of 5.56% correctly reclassified. The NRI value was 13.46%, with a z value of 1.34 and a p value of 0.18. Compared with model 1, the overall predictive ability of model 2 also improved; for patients with CALs, the average predictive probability for CALs was increased by 1.94%; for patients without CALs, the average predictive probability for non-CALs (NCALs) was increased by 3.06%. The IDI value was 5.00%, with a z value of 2.26 and a p value of 0.02.

DISCUSSION

KD is one of the most frequently acquired heart diseases in the world.[22] Its incidence has increased over recent decades in many countries, including Japan, Korea, and China.[23–25] The most severe complication or sequela is the formation of CALs, which may subsequently result in long-term sequelae, such as stenosis, obstruction and myocardial infarction.[26 27] Therefore, it is important to identify and use risk factors to predict the incidence of KD with CAL. High-risk children can be identified in the diagnosis of KD.

In this study, we found that the levels of fever duration, PLT count, CRP, and ESR were significantly higher in the CAL group than those in the NCAL group. ALB was

Table 3 Logistic regression analysis of risk factors for KD with CALs

Variables	β	Wald	P value	OR	95% CI
Male	0.826	2.204	0.138	2.284	(0.768 to 6.793)
Fever duration	0.173	5.179	0.023	1.162	(1.046 to 1.352)
IKD	−0.921	2.902	0.088	0.398	(0.138 to 1.149)
PLT	0.006	9.486	0.005	1.006	(1.002 to 1.010)
ALB	−0.143	7.607	0.006	0.866	(0.782 to 0.959)
CRP	0.020	10.131	0.001	1.020	(1.008 to 1.033)
ESR	0.025	11.914	0.002	1.032	(1.014 to 1.051)
MMP-9	0.001	4.836	0.022	1.001	(1.001 to 1.002)
Constant	−0.754	0.175	0.676	0.470	–

ALB, albumin; CAL, coronary artery lesion; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IKD, incomplete Kawasaki disease; KD, Kawasaki disease; MMP-9, matrix metalloproteinase-9; PLT, platelet.

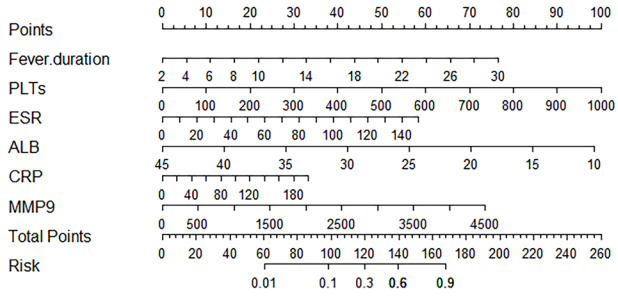

Figure 1 The nomogram for personal risk probability of Kawasaki disease (KD) with coronary artery lesion (CAL). Find the predictor points on the uppermost point scale that correspond to each patient variable and add them up. The total points projected to the bottom scale indicate the % probability of KD with CALs. ALB, albumin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MMP9, matrix metalloproteinase-9; PLT, platelets.

significantly lower in the CAL group than that in the NCAL group. Male patients and patients with IKD were more common in patients with CALs. The results of multivariable logistic regression analysis suggested that longer fever; elevated CRP, ESR, and PLT; and low ALB levels were independent risk factors for KD with CAL, which is consistent with the results of the previous studies.[28–30] In addition to these traditional independent risk factors, the most remarkable finding was the relationship between MMP-9 on admission and CAL in patients with KD. MMP-9 levels in the CAL group were significantly higher than those in the NCAL group ($p<0.05$). A Chinese meta-analysis performed

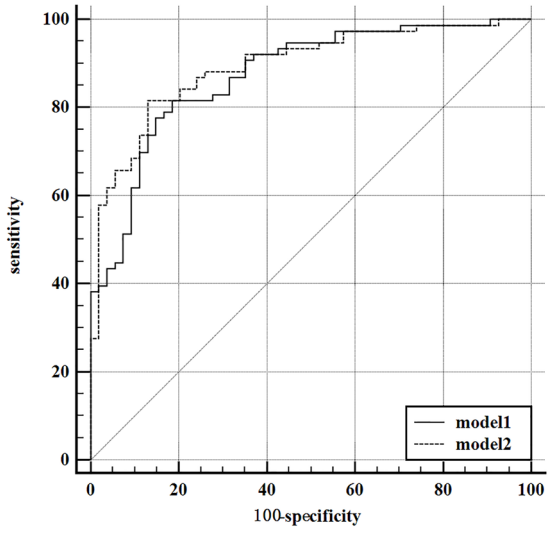

Figure 2 Receiver operating characteristic (ROC) curves for models with and without matrix metalloproteinase-9 (MMP-9). Prediction model 1 (AUC was 0.87, sensitivity was 81.58%, specificity was 81.48%) was constructed based on traditional independent risk factors (fever duration time, PLT, ALB, CRP, ESR), and MMP-9 was added on the basis of traditional risk factors to construct prediction model 2 (AUC was 0.89, sensitivity was 81.53%, specificity was 87.04%). ALB, albumin; AUC, area under the curve; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PLT, platelet.

by Ying *et al* on 993 patients from 19 studies demonstrated that MMP-9 levels in patients with CAL were higher than that of patients with NCAL.[18] Zeng analyzed 68 cases of KD (36 cases with CALs) and found that the levels of MMP-9 in the serum were significantly increased in the acute phase of KD, especially in the CAL group.[19] Based on the correlation between MMP-9 levels and CALs, it was suggested that MMP-9 may potentially be a predictor of CALs in patients with KD. However, few studies have reported the predictive ability of MMP-9 for KD combined with CALs. In this study, we found that MMP-9 is an independent risk factor for CALs after adjustments for other variables (male, fever duration, IKD, PLT, ALB, CRP, ESR). However, the beta-coefficient for MMP-9 in the model was only 0.001 in our study. This indicates that MMP-9 is a weak independent predictor of CALs.

MMP-9 belongs to a family of MMPs and has specific collagenase and elastase activity. It can degrade the subendothelial layer mainly composed of collagen tissue, elastin and proteoglycan.[31 32] Some studies have found that MMP-9 also helps inflammatory cells such as leucocytes, T lymphocytes, and monocyte macrophages to move deeper into the blood vessel wall, allowing damage to develop deeper into the blood vessel wall.[33] Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an active inhibitor of MMP-9 and binds to MMP-9 in a 1:1 ratio to form a complex, which blocks the binding of MMP-9 to the substrate and makes it inactive.[14 34] Under physiological conditions, MMP-9 and TIMP-1 are in dynamic balance. At present, vasculitis damage caused by high activation of the immune system in children with KD has been recognized. Many studies have shown that inflammatory cells such as white cells, central granulocytes, and monocytes/macrophages are activated and secrete a large amount of MMP-9 in the acute phase of children with KD.[35–37] In our research, we found that patients with higher MMP-9 had higher white cells and NE% values, suggesting that circulating white cells and neutrophils may be a source of the MMP-9 secreted into the circulation. However, MO% was lower in patients with higher MMP-9. Sakata *et al* performed immunohistological examination of CALs from a patient who died of KD in the acute phase and found that there was no MMP-9 mRNA elevation in mononuclear cells.[12] Some studies have shown that these activated inflammatory cells secrete a large number of inflammatory factors, such as tumor necrosis factor-α, IL-6, and IL-1 (β).[38 39] It was found that these inflammatory factors could upregulate the expression of MMP-9. Therefore, the dynamic balance between MMP-9 and TIMP-1 was destroyed, and the vascular wall matrix was degraded, which further caused the migration of inflammatory cells to the deep blood vessels, resulting in the reconstruction of vascular structure. Nevertheless, there was no significant difference in IL-6 between the higher MMP-9 group and the lower MMP-9 group in the present study. In addition, according to our findings, CRP and ESR were higher in patients with higher MMP-9 levels. This suggests that elevated MMP-9 on admission indicates a severe inflammatory process.

In this study, we constructed a predictive model 1 using traditional independent risk factors, with an AUC of 0.87. A new risk factor MMP-9 was added to the traditional risk factors to construct a predictive model 2, and the AUC was

0.89. Compared with model 1, the AUC of model 2 only improved by 0.02. Wang *et al* showed that the addition of a new risk factor to a set of standard risk factors predicting cardiovascular disease increases the model AUC from 0.76 to 0.77, which is an increase of only 0.01.[40] Ware and Pepe *et al*[41 42] show simple examples in which enormous ORs are required to meaningfully increase the AUC. Therefore, simply using the improvement of the AUC to evaluate the predictive value of new risk factors is not sensitive in many cases, and its significance is not easy to understand and difficult to translate into appropriate clinical interpretation. The application of NRI and IDI provides a superior method for analyzing the incremental value of a new risk factor.[43] In our study, we found that the addition of MMP-9 to the model 1 actually results in more patients being reclassified. The NRI was 13.46%, which means 13.46% of the patients were correctly reclassified (7.90% correct reclassifications for patients with CALs, 5.56% correct reclassifications for patients without CALs), but this small difference was not statistically significant ($p>0.05$). The IDI was 5.00%, which was statistically significant ($p<0.05$), which means that after the addition of MMP-9, the overall predictive ability of model 2 improved, and the ability to comprehensively discriminate was increased by 5.00% (for patients with CALs, the average predictive ability for CALs was increased by 1.94%; for patients without CALs, the average predictive ability for NCALs was increased by 3.06%). This indicates that the major benefit of adding MMP-9 to traditional risk factors is that can make a more accurate prediction of CALs/NCALs early in a patient's treatment for further individualized treatment. For predicted CALs, prompt treatment with high-dose (2 g/kg) IVIG could significantly reduce incidence of CALs; for predicted NCALs, unnecessary treatment-related toxicities can be avoided. In addition, Hilden pointed out that the selection of predictors requires a comprehensive consideration of public health needs.[44] Our findings are mainly based on the serum detection of MMP-9, which may be a safer, cheaper and more valid method to detect the expression of MMP-9, and easy to accept by patients. It might be worth including serum MMP-9 in the predictive model.

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Contributors XH and LW originally conceived the study. LW and YY conducted the experiment. QC and YC contributed to data acquisition. LW, QC, YC, QL, XC, CW, and PQ contributed to data analysis and statistical analyses. LW drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Parental/guardian consent obtained.

Ethics approval This study is a retrospective study, which only collected the patient's clinical data and laboratory data, and all patient information remained confidential. The Ethics Committee of Lanzhou University Second Hospital (No 2018A-059) waived the requirement for informed consent. In addition, the blood sample used in this study is the remaining blood sample from other studies. The informed consent signed by the patient's parent or guardian has clearly stated that the blood sample can be used for subsequent clinical research.

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