

Role of Pyroptosis in IVIG-Resistant Kawasaki Disease and the Establishment of a New Predictive Model

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Background: Intravenous immunoglobulin (IVIG) resistance may be an increased risk of coronary artery lesions which is the serious complication of Kawasaki disease (KD). Early and accurate identification of IVIG-resistant patients has an important clinical value.

Objective: To establish a new predicting model by detecting the pyroptosis markers with other clinical indicators.

Methods: A total of 144 children with KD who were hospitalized in Wuhan Children's Hospital from January 2022 to December 2022 were enrolled in this prospective study, among whom 120 had IVIG-sensitive KD and 24 had IVIG-resistant KD. *NLRP3*, *ASC*, *CASP1*, and *GSDMD* were quantified in peripheral blood cells of all children by using Real-time Quantitative Polymerase Chain Reaction (QRT-PCR) assay. The enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of cytokines like IL-1 β and IL-18. Logistic regression analysis was performed to identify independent risk factors for resistance to IVIG in children with KD. These meaningful variables were assigned values based on odds ratios and became components of the new risk assessment model. The prediction efficiency of this model was tested and evaluated based on the receiver operating characteristic (ROC) curves.

Results: 1. IVIG-resistant KD group had significantly lower mRNA expression of *CASP1* than IVIG-sensitive KD group ($P<0.05$). 2. IVIG-resistant KD group had significantly higher level of serum procalcitonin (PCT) and tumor necrosis factor- α (TNF- α) and lower level of serum sodium (Na) than the IVIG-sensitive KD group ($P<0.05$). 3. The assessment model had a sensitivity of 91.7% and a specificity of 69.2% in the prediction of IVIG-resistant KD ($P<0.001$).

Conclusion: Combined examination by *CASP1* and above laboratory indexes has clinical practical value for the diagnosis of IVIG-resistant KD.

Keywords: Kawasaki disease, resistance to intravenous immunoglobulin, *CASP1*, child

Introduction

Kawasaki disease (KD) is an acute febrile vasculitis syndrome that predominantly affects medium size arteries, especially the coronary arteries, which is a common cause of acquired heart disease in children world-wide.¹ At present, the clinical efficacy of high dose intravenous immunoglobulin (HD-IVIG) in combination with aspirin has been affirmed fully, which can be helpful to the alleviation of acute symptoms and obviously decrease the risk of coronary artery lesion (CAL).² However, 15–20% of the patients who received the first IVIG infusion may do not respond to it, and have persistent fever or recrudescent fever at least 36 hours after completion of the initial therapy.³ Studies have shown that initial IVIG resistance is associated with increased risks of CAL and severe complications such as the Kawasaki Disease Shock Syndrome (KDSS) or KD-MAS (KD-Macrophage activation syndrome).^{4,5} Predicting the effectiveness of initial treatment early can help identify children who are at high risk for CAL and improve the prognosis of patients significantly.

Pyroptosis is a newly discovered programmed cell death, which relies on caspase activation, that in turn stimulates an inflammatory cascade reaction and might cause tissue damage.⁶ Previous researches have shown that pyroptosis plays

a significant role in several autoimmune diseases,⁷ and the expression of pyroptosis markers was notably upregulated in KD in acute period.^{8–10} However, there are few studies about how they work in IVIG unresponsiveness. In this study, we discussed the expression and role of signaling molecules in the canonical pathway of pyroptosis in IVIG-resistant KD, and established a new predicting model of IVIG-resistant KD by detecting the pyroptosis markers with other clinical indicators, which might provide several new methods of the diagnosis and treatment of IVIG-resistant KD.

Materials and Methods

Subjects Studied

We enrolled 144 KD patients in our department between January 2022 and December 2022. There were 89 boys and 55 girls, and the mean age was 2.42 ± 1.04 years. Inclusive criteria: (1) patients aged 6 months to 5 years old; (2) patients diagnosed according to the criteria published by the American Heart Association (AHA) in 2017;¹¹ (3) patients received standard treatment with a single dosage of IVIG treatment (2g/kg) and oral aspirin (30–50 mg/kg·d) in the acute phase of illness; (4) the diagnostic criteria of IVIG-resistant KD: persistent or recrudescent fever at least 36 hours after completion of the first IVIG infusion. Exclusive criteria: (1) recurrent KD; (2) patients who have used hormone drugs during early stage of illness; (3) patients who did not receive a single dosage of IVIG treatment (2g/kg) during the course; (4) patients had a severe infection, chronic disease, or other complications which can affect the therapeutic prognosis for illness; (5) patients with missing clinical or laboratory information.

Methods

Samples Collection

We collected blood samples from KD patients before the initial IVIG treatment. Serum was separated from the blood by centrifugal method and were stored at -80°C until analysis. Total RNA was extracted from white blood cells using Trizol reagent (Takara) for the next experimental step.

Real-Time Quantitative PCR

The solution system of reverse transcription was prepared following the instruction of test kits (Vazyme, China). We designed the primers to amplify the target genes on the Premier 5 platform, as shown in Table 1. PCR was performed with ChamQ SYBR qPCR Master Mix (Vazyme), and the relative expression of genes were calculated using $2^{-\Delta\Delta\text{Ct}}$.

Serum Cytokine Level Measurements

Serum levels of IL-1 β and IL-18 were measured by enzyme-linked immunosorbent assays with the instructions of test kits (HYcezmBio, China). The detailed steps in this process were as follows: (1) All components should be reheated for at least 60 minutes to make sure that they are fully reheated to room temperature; (2) Concentrated wash buffer and distilled water were diluted in proportion of 1:20; (3) Substrate solution A and B were mixed thoroughly; (4) Standard, sample

Table 1 Primers for Genes

Gene Symbol	Primers (5' to 3')
NLRP3	F: GATCTTCGCTGCGATCAACAG R: CGTGCATTATCTGAACCCAC
ASC	F: CCTACTGTTCTTTCTGTGGGAAG R: CGAGGTCGTCAGCCATCAC
CASPI	F: TTTCCGCAAGGTTTCGATTTTCA R: GGCATCTGCGCTCTACCATC
GSDMD	F: GTGTGTCAACCTGTCTATCAAGG R: CATGGCATCGTAGAAGTGAAG
GAPDH	F: GGTGAAGGTCGGAGTCAACGG R: GGTCAATGAGTCCTTCCACGATCATACC

diluent, and sample were added to the corresponding holes, respectively; (5) Detection antibody which marked by HRP was added to standard hole, 0 value hole, and sample hole; (6) Plates were incubated in water bath for 60 minutes at 37°C; (7) Plates were washed and patted dry for 5 times; (8) Substrate mixture was added to each hole, and plates were incubated in water bath for 15 minutes at 37°C; (9) Stop solution was added to each hole; (10) Optical density of each hole were read by the microplate reader.

Data Collection

A total of 144 children diagnosed with KD were included according to inclusive and exclusive criteria during the study (Figure 1). All the KD patients received a single dosage of IVIG treatment (2g/kg) as initial therapy and were divided into two groups based on response to treatment above-mentioned. All cases have complete average materials (gender and age) and clinical manifestations (days of illness, rash, conjunctivitis, lip and tongue changes, lymphadenopathy, extremity changes, and echocardiographic features (with or without coronary artery lesion)). Laboratory data including white blood cell count (WBC), hemoglobin (HB), platelet count (PLT), percentage of neutrophilic granulocyte (NEUT%), C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR), serum ferritin (FERR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase-MB (CK-MB), lactic dehydrogenase (LDH), total bilirubin (TB), conjugated bilirubin (CB), serum creatinine (Scr), urea nitrogen (BUN), serum sodium (Na), serum chlorine (Cl), serum potassium (K), serum calcium (Ca), CD3+CD4+T-lymphocyte count, CD3+CD8+T-lymphocyte count, immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and tumor necrosis factor- α (TNF- α) were obtained.

Statistical Analysis

SPSS 24.0 statistical analysis package was used for data processing and statistical analysis. Measurement data in normal distribution were presented as mean $\pm$ SD and two sample *t* test and one-way ANOVA were applied for differences comparison among groups. Measurement data in abnormal distribution were presented as median (25th and 75th percentile) [M(Q1, Q3)], differences between groups were compared through analysis of Mann–Whitney *U*-test. Count data were presented as the number of cases and compared among groups by χ^2 test. The variables that differed between groups were analyzed by logistic regression analysis and were assigned scores based on odds ratios to establish a new assessment model. ROC curves were used to evaluate the prediction efficiency of this new predictive model.

Results

Comparisons of Expression of Pyroptosis Markers Between IVIG-Sensitive Group and IVIG-Resistant Group

Compared with the IVIG-sensitive KD group, the levels of *NLRP3* and *CASP1* were degraded in IVIG-resistant KD group ($P<0.05$). There were no significant difference in levels of *ASC*, *GSDMD*, IL-1 β , and IL-18 between these two KD groups ($P>0.05$) (Table 2).

Clinical and Laboratory Data in IVIG-Sensitive Group and IVIG-Resistant Group

Comparisons of Clinical Data Between IVIG-Sensitive Group and IVIG-Resistant Group

As shown in Table 3, there were no significant differences in sex, age, and days of illness between the two KD groups ($P>0.05$). Meanwhile, compared with responders to IVIG treatment, the proportion of main clinical manifestations such as rash, changes of lips and tongue, adenopathy, changes of hands and feet, and coronary artery lesion was elevated in non-responders, but the difference was not statistically significantly ($P>0.05$).

Comparisons of Laboratory Data Between IVIG-Sensitive Group and IVIG-Resistant Group

Compared with the IVIG-sensitive group, laboratory indicators including PCT and TNF-A were significantly increased in the IVIG-resistant group ($P<0.05$), while the concentration of Na in the non-responders to initial therapy decreased

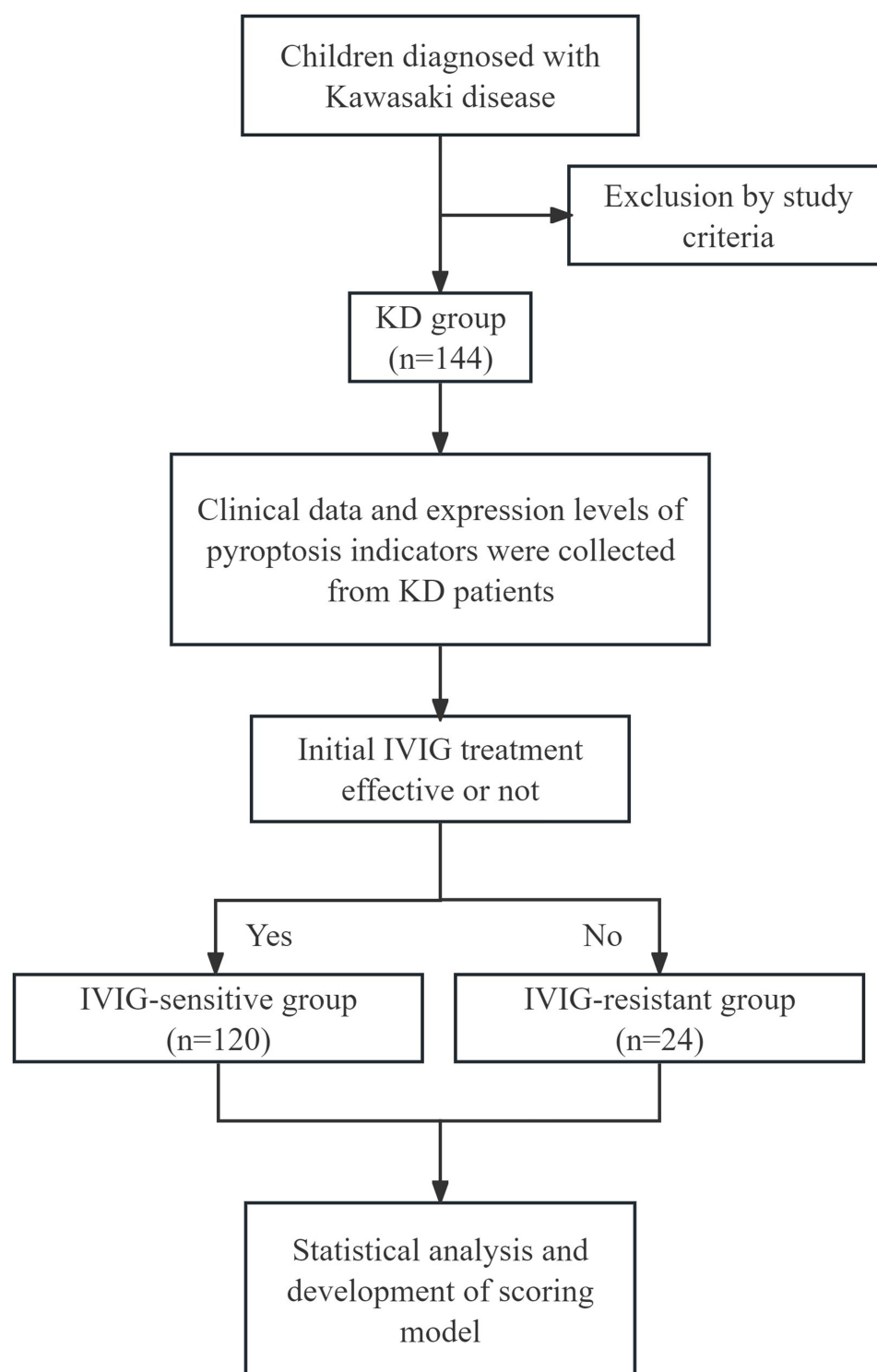


Figure 1 Flow chart of study subject inclusion.

Abbreviations: KD, Kawasaki disease; IVIG, Intravenous immunoglobulin.

substantially ($P < 0.001$). There were no statistical differences between the two groups on WBC, Hb, PLT, NEUT%, CRP, ESR, FERR, ALT, AST, CK-MB, LDH, TB, CB, Scr, BUN, Cl, K, Ca, CD3⁺CD4⁺T, CD3⁺CD8⁺T, IgA, IgM, IgG, IL-2, IL-4, IL-6, IL-8 and IL-10 ($P > 0.05$) (Table 4).

Table 2 Comparisons of Expression of Pyroptosis Markers Between IVIG-Sensitive Group and IVIG-Resistant Group

	IVIG-sensitive KD	IVIG-resistant KD	Z	P value
No. of patients	120	24		
NLRP3	0.92 (0.66, 1.15)	0.53 (0.39, 1.34)	-2.444	0.015*
ASC	0.61 (0.49, 0.90)	0.40 (0.33, 0.59)	-1.849	0.064
CASPI	2.38 (1.80, 3.44)	1.79 (1.17, 2.14)	-3.184	0.001**
GSDMD	1.31 (0.85, 1.97)	1.16 (0.85, 1.59)	-0.713	0.476
IL-1 β (pg/mL)	14.41 (12.13, 16.72)	12.94 (10.38, 16.32)	-1.324	0.185
IL-18 (pg/mL)	57.87 (50.90, 69.22)	54.58 (46.37, 68.35)	-1.238	0.216

Note: *P value of <0.05; **P value of <0.01.

Table 3 Comparisons of Clinical Data Between IVIG-Sensitive Group and IVIG-Resistant Group

Clinical data	Total	IVIG-sensitive KD	IVIG-resistant KD	X ² /z	P value
No. of patients	144	120	24		
Sex(male/female)	89 /55	74 /46	15/9	0.006	0.939
Age (years), mean $\pm$ SD	2.50 (1.82,3.21)	2.50 (1.82,3.19)	2.64 (1.84,3.71)	0.289	0.772
Days of illness(d), median(Q1,Q3)	5 (4,7)	5 (4,7)	6 (5,8)	1.891	0.059
Rash, n(%)	112 (77.8%)	92 (76.7%)	20 (83.3%)	2.937	0.087
Conjunctivitis, n(%)	128 (88.9%)	109 (90.9%)	19 (79.2%)	2.756	0.097
Changes of lips and tongue, n(%)	124 (86.1%)	102 (85.0%)	22 (91.1%)	0.743	0.387
Adenopathy, n(%)	105 (72.9%)	84 (70.0%)	21 (87.5%)	3.102	0.078
Changes of hands and feet, (n(%))	66 (45.8%)	53 (44.2%)	13 (54.2%)	0.806	0.369
CAL, n(%)	15 (10.4%)	10 (8.3%)	5 (20.8%)	3.349	0.067

Table 4 Comparisons of Laboratory Data Between IVIG-Sensitive Group and IVIG-Resistant Group

Laboratory data	IVIG-sensitive KD	IVIG-resistant KD	Z/t	P value
WBC ($\times 10^9/L$)	10.81 (8.10, 15.39)	9.53 (7.54, 18.39)	-0.088	0.930
Hb (g/L)	105.97 $\pm$ 12.53	104.63 $\pm$ 14.59	0.509	0.615
PLT ($\times 10^9/L$)	331.5 (251.50, 408.00)	401.00 (237.25, 630.75)	1.447	0.148
NEUT%	65.6 (52.50, 75.80)	60.80 (45.80, 81.80)	-0.155	0.876
CRP (mg/L)	67.85 (31.45, 97.00)	40.80 (17.65, 104.20)	-1.380	0.167
PCT (ng/mL)	0.44 (0.22, 1.24)	5.21 (1.64, 10.14)	5.624	<0.001***
ESR (mm/h)	41.50 (20.00, 74.00)	58.00 (16.25, 88.50)	0.434	0.664
FERR (ng/mL)	170.75 (115.4, 233.11)	208.21 (130.68, 278.92)	0.237	0.237
ALT (U/L)	22.00 (13.00, 82.00)	18.50 (14.00, 49.75)	-0.075	0.940
AST (U/L)	34.50 (22.00, 53.75)	34.00 (28.00, 44.50)	0.330	0.742
CK-MB (U/L)	25.50 (21.25, 28.43)	26.00 (17.00, 29.50)	-1.266	0.206
LDH (U/L)	289.00 (249.25, 403.50)	289.00 (252.50, 383.25)	-0.638	0.523
TB (μ mol/L)	6.15 (4.33, 10.80)	8.20 (3.18, 11.95)	0.088	0.930
CB (μ mol/L)	2.70 (1.93, 4.58)	3.70 (1.90, 5.63)	0.590	0.555
Scr (μ mol/L)	25.00 (21.25, 28.43)	22.30 (21.23, 26.65)	-1.263	0.207
BUN (mmol/L)	3.30 (2.70, 4.18)	3.05 (2.53, 4.45)	0.064	0.949
Na (mmol/L)	136.28 $\pm$ 3.08	134.51 $\pm$ 1.51	5.023	<0.001***
Cl (mmol/L)	100.63 $\pm$ 3.46	99.33 $\pm$ 4.19	1.722	0.096
K (mmol/L)	4.35 (3.76, 4.79)	4.40 (3.70, 5.08)	0.298	0.766
Ca (mmol/L)	2.27 (2.17, 2.36)	2.24 (2.17, 2.38)	0.110	0.912

(Continued)

Table 4 (Continued).

Laboratory data	IVIG-sensitive KD	IVIG-resistant KD	Z/t	P value
CD3 ⁺ CD4 ⁺ T (/uL)	1051.00 (650.00, 1505.00)	996.50 (492.75, 1369.25)	−0.694	0.488
CD3 ⁺ CD8 ⁺ T (/uL)	656.00 (447.00, 914.00)	461.50 (249.75, 955.25)	−1.394	0.163
IgA (g/L)	0.76 (0.58, 1.03)	0.87 (0.65, 1.42)	1.438	0.151
IgM (g/L)	1.07 (0.81, 1.43)	1.15 (0.91, 1.67)	1.232	0.218
IgG (g/L)	7.29 (5.56, 9.90)	11.00 (5.51, 21.30)	1.542	0.123
IL-2 (pg/mL)	3.30 (1.66, 4.12)	3.84 (2.01, 5.57)	1.135	0.256
IL-4 (pg/mL)	2.86 (1.83, 4.19)	2.82 (1.80, 3.99)	−0.298	0.765
IL-6 (pg/mL)	78.55 (30.05, 161.93)	88.40 (24.00, 333.40)	0.229	0.819
IL-8 (pg/mL)	439.23 (58.38, 3139.62)	273.24 (87.55, 1497.44)	−0.409	0.682
IL-10 (pg/mL)	12.39 (6.81, 27.61)	19.96 (6.63, 49.42)	1.016	0.309
TNF-α (pg/mL)	4.71 (3.53, 7.11)	6.95 (4.50, 10.41)	2.300	0.021*

Notes: *P value of<0.05; ***P value of<0.001.

Development of a New Predicting Model of IVIG Resistance in KD

Univariate and multivariate regression analyses were performed to assess the variables which were significantly different between the two KD groups. As shown in Table 5, results indicated that PCT, Na, and *CASPI* may be important risk factors for IVIG-resistant KD. ROC curves (Figure 2) showed the AUC of PCT, Na, *CASPI*, and the new scoring model composed of the above three indicators was 0.804, 0.742, 0.706, and 0.899 (*P*<0.05). The cutoff value of PCT for the prognosis of IVIG-resistant KD was 2.49 ng/mL with the sensitivity of 75% and specificity of 85.8%. The sensitivity and specificity were 98.5% and 50% with a cutoff of Na ≤ 136.65mmol/L. Moreover, the sensitivity and specificity were 70.8% and 69.2% with a cutoff of *CASPI* ≤ 2.1472. The sensitivity of the new scoring model was 91.7%, and the specificity was 69.2% with a cutoff of point ≤1.5 (Table 6).

Discussion

The clinical efficacy of HD-IVIG with aspirin, which known as first-line treatment, has been fully affirmed in clinical practice. It has been proved to relieve clinical symptom and reduce the incidence of CAL effectively.¹² However, some children who are resistant to the initial therapy tend to suffer more severe complications even result in a threat to life when the condition gets worse.¹³ Therefore, how to early predict the response to this treatment and improve the diagnosing efficiency should be solved in advance. At present, several scoring systems for predicting IVIG resistance (Kobayashi scoring system, Egami scoring system, and Sano scoring system, for instance) have been shown to have limited prediction efficiency for non-Japanese population.¹⁴ The purpose of this study was to develop a new predictive scoring system for Chinese population by combining expression of pyroptosis markers with main clinical indicators.

Table 5 Univariate and Multivariate Regression Analysis of Variables Differ Between IVIG-Sensitive Group and IVIG-Resistant Group

Variable	Univariate		Multivariate	
	OR (95% CI)	P value	OR(95% CI)	P value
PCT (ng/mL)	1.543 (1.277, 1.865)	<0.001***	1.484 (1.227, 1.794)	<0.001***
Na (mmol/L)	0.776 (0.665, 0.919)	0.003**	0.799 (0.643, 0.993)	0.043*
TNF-α (pg/mL)	1.002 (0.982, 1.022)	0.842		
NLRP3	0.849 (0.342, 2.109)	0.724		
CASPI	0.578 (0.384, 0.871)	0.009**	0.572 (0.338, 0.967)	0.037*

Notes: *P value of<0.05; **P value of<0.01;***P value of<0.001.

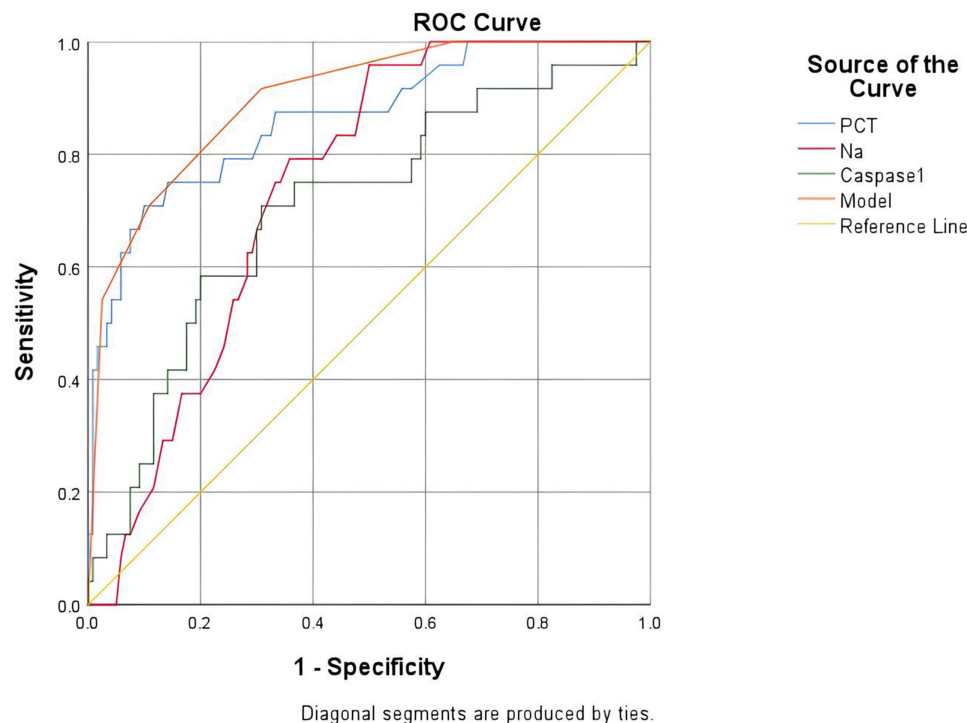


Figure 2 ROC curves of PCT, Na, CASP1 and new scoring model for predicting IVIG resistance. The power of various indicators and new scoring model of prediction for IVIG-resistance was tested with the ROC curves: PCT: AUC: 0.864 (95% CI, 0.779–0.949); Na: AUC: 0.742 (95% CI, 0.657–0.826); CASP1: AUC: 0.706 (95% CI, 0.590–0.823); The new scoring model: AUC: 0.899 (95% CI, 0.835–0.963).

Abbreviations: ROC, Receiver operating characteristic; AUC, Area under curve.

The *CASP-1*-mediated canonical signaling pathway is accompanied by the activation of *GSDMD* and the release of pro-IL-1 β and pro-IL-18.¹⁵ When microbial infections and non-microbial diseases happen, the assembly of canonical inflammasomes occurs in response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), followed by the activation of downstream signaling pathway and result in a series of inflammatory changes.¹⁶ Pyroptosis has been confirmed to play a role in the pathogenesis of vascular inflammation of KD. Anzai et al reported that *CASP1* and IL-1 β might participate in the coronary arteritis induced by *Candida albicans* water-soluble fraction and the inflammatory reaction was protected against in *NLRP3*^{−/−} and *ASC*^{−/−} mice.¹⁷ Lee et al also reported that LCWE-induced vasculitis was inhibited in *CASP1*^{−/−} and *IL-1R*^{−/−} mice.¹⁸ Furthermore, Chang Jia et al found that MCC950 can decrease the expression of *NLRP3*, *CASP1*, *GSDMD*, IL-1 β , and IL-18 in CAW-induced vasculitis model as a direct *NLRP3* inhibitor and promote the viability of mice vascular endothelial cells significantly.⁹ In conclusion, the canonical pathway mediated by *CASP1* correlates closely with the development of KD vasculitis and might provide a new thought for the therapeutic strategy in the future.

As an important protease in pyroptosis, *CASP1* can be activated by a variety of inflammasomes and trigger a serial cascade of immune reactions.¹⁹ Previous research has shown that the up-regulation of *CASP1* in the acute stage of KD might be related to the development of vasculitis.^{9,10} The results of our study show that: (1) The lower mRNA expression

Table 6 Optimal Cut-off Points and Assignments of Variables in the Predicting Model

Variable	AUC	Cut-off point	Sensitivity	Specificity	Point	95% CI	p value
PCT (ng/mL)	0.864	2.49	0.750	0.858	2(≥ 2.49), 0(<2.49)	0.779–0.949	<0.001***
Na (mmol/L)	0.742	136.65	0.958	0.500	1(≤ 136.65), 0(>136.65)	0.657–0.826	<0.001***
CASP1	0.706	2.1472	0.708	0.692	1(≤ 2.1472), 0(>2.1472)	0.590–0.823	0.001**
Model	0.899	1.5	0.917	0.692	High risk(≥ 1.5), Low risk(<1.5)	0.835–0.963	<0.001***

Notes: **P value of<0.01;***P value of<0.001.

was found in the IVIG-resistant group than IVIG-sensitive group ($P=0.001$); (2) Multivariate regression analysis showed that *CASP1* could be a risk factor for IVIG unresponsiveness ($P<0.001$). The above-mentioned results indicate that *CASP1* might play a role in the pathophysiology in IVIG-resistant KD, but the exact mechanism remains unclear and needs to be studied further.

In this study, we also found that the expression level of *NLRP3* mRNA were declined notably in the IVIG-resistant group in comparison with that in the IVIG-sensitive group ($P<0.05$). However, there was no significant difference between the two groups on the mRNA levels of *ASC*, *GSDMD*, IL-1 β , and IL-18 ($P>0.05$). These findings are similar to those in previous studies.^{10,20}

At present, the specific pathogenesis of KD still remains unknown. Most studies put forward that the development of KD is closely related to genetic predisposition, immune mechanism, and infectious factor,²¹ often with accompanying inflammatory cascade reactions, and IVIG-resistant patients are thought to have severe inflammatory response.²² So we initially assumed that compared with the IVIG-sensitive group, the mRNA levels of inflammatory factors such as *NLRP3* and *CASP1* in the IVIG-resistant group might significantly increase. However, previous studies did not provide enough evidence for it and the results of our study refute this hypothesis. There are no literatures that are published to discuss the causes of this problem and future research is needed to analyze and solve them.

The pathogenesis of KD combining with hyponatremia is believed to involve increased ADH secretion, increased activity of natriuretic peptide, and renal salt wasting caused by renal involvement.²³ For the existing predictive models for IVIG unresponsiveness in patients with KD, the threshold value of serum sodium concentrations ranged from 133 to 135.2 mmol/L^{22,24–26} and the higher value in our study might be related to different subjects, region factors, and sampling time. Procalcitonin has been widely proved to be a significant biomarker to predict the risk of IVIG resistance in KD. Previous studies suggested that the threshold value of procalcitonin concentrations ranged from 0.25–4.3ng/mL,^{21,27–29} which were consistent with the results of our research□

As shown in Figure 2, the prediction efficiency of multivariate factors model was better than that of the single-factor model. The study was aimed to develop a new predictive model for IVIG-resistant KD through a combination of three indicators. The scoring methods were as follows: $PCT \geq 2.49$ (2 points), $Na \leq 136.65\text{mmol/L}$ (1 point), $CASP1 \leq 2.1472$ (1 point); 0–1.5 points: low risk for IVIG-resistant, ≥ 1.5 points: high risk for IVIG-resistant. Applied to this group of study subjects: sensitivity 91.7%, specificity 69.2%, AUC: 0.899. However, we conducted the study with small sample size at a single center, which might influence the accuracy and reliability of the experiment results. Multi-center and large-scale studies should be developed to confirm the effectiveness of this new scoring model further.

Limitations of our research were as follows: (1) This was a single-center study and the sample size of each group was small; (2) The predictive efficacy of the model was only evaluated in the experimental group; (3) Peripheral white blood cells were used for RNA extraction, which might affect the accuracy of the experimental results; (4) The experimental method to detect the expression of pyroptosis-related genes was very single; (5) This study only detected pyroptosis markers before IVIG treatment due to the lack of clinical data of patients after treatment. (6) We focused solely on the canonical pyroptosis pathway gene. Other genes like *CASP 3* and *CASP 4* were also involved in the pathogenetic process of KD, and *CASP 3* was demonstrated to be associated with IVIG-resistance;^{10,30} (7) The detection of *CASP1* had strict requirement to its device.

In conclusion, pyroptosis may be involved in the course of KD and is associated with the IVIG resistance. The application of this scoring model will help predict the IVIG-resistant KD and provide a new thought for the diagnosis and treatment of KD.

Ethics Approval

This study has been approved by the Wuhan Children's Hospital Institutional Review Board (NO.2022R053-E01). Our study complied with the Declaration of Helsinki.

Consent to Participate

We obtained written informed consent from the guardians of all participants.

Consent for Publication

This study didn't involve the patient's personal privacy.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests.

References

1. Butters C, Curtis N, Burgner DP. Kawasaki disease fact check: myths, misconceptions and mysteries. *J Paediatr Child Health*. 2020;56(9):1343–1345. doi:10.1111/jpc.15101 Epub 2020 Aug 8. PMID: 32770807.
2. Sosa T, Brower L, Divanovic A. Diagnosis and management of Kawasaki disease. *JAMA Pediatr*. 2019;173(3):278–279. doi:10.1001/jamapediatrics.2018.3307 PMID: 30667467.
3. Li X, Chen Y, Tang Y, et al. Predictors of intravenous immunoglobulin-resistant Kawasaki disease in children: a meta-analysis of 4442 cases. *Eur J Pediatr*. 2018;177(8):1279–1292. doi:10.1007/s00431-018-3182-2 Epub 2018 Jun 8. PMID: 29948255; PMCID: PMC6061038.
4. Cai JH, Tang M, Zhang HX, et al. Therapeutic window of intravenous immunoglobulin (IVIG) and its correlation with IVIG-resistant in Kawasaki disease: a retrospective study. *J Pediatr*. 2023;99(2):161–167. doi:10.1016/j.jpeds.2022.07.003 Epub 2022 Aug 20. PMID: 35995125; PMCID: PMC10031361.
5. Zhang RL, Lo HH, Lei C, et al. Current pharmacological intervention and development of targeting IVIG resistance in Kawasaki disease. *Curr Opin Pharmacol*. 2020;54:72–81. Epub 2020 Sep 18. PMID: 32956895; PMCID: PMC7500898. doi:10.1016/j.coph.2020.08.008
6. Wei X, Xie F, Zhou X, et al. Role of pyroptosis in inflammation and cancer. *Cell Mol Immunol*. 2022;19(9):971–992. doi:10.1038/s41423-022-00905-x Epub 2022 Aug 15. PMID: 35970871; PMCID: PMC9376585.
7. You R, He X, Zeng Z, et al. Pyroptosis and its role in autoimmune disease: a potential therapeutic target. *Front Immunol*. 2022;13:841732. doi:10.3389/fimmu.2022.841732
8. Wang Z, Wang Q, Jin J, et al. The diagnostic role of AIM2 in Kawasaki disease. *Clin Exp Med*. 2021;21(1):41–47. doi:10.1007/s10238-020-00669-6
9. Jia C, Zhang J, Chen H, et al. Endothelial cell pyroptosis plays an important role in Kawasaki disease via HMGB1/RAGE/cathepsin B signaling pathway and NLRP3 inflammasome activation. *Cell Death Dis*. 2019;10(10):778. doi:10.1038/s41419-019-2021-3
10. Kuo KC, Yang YL, Lo MH, et al. Increased expression of pyroptosis in leukocytes of patients with Kawasaki disease. *Diagnostics*. 2021;11(11):2035. doi:10.3390/diagnostics11112035 PMID: 34829381; PMCID: PMC8620614.
11. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association [published correction appears in circulation. *Circulation*. 135(17):e927–99. doi:10.1161/CIR.0000000000000484
12. Wang S, Ding C, Zhang Q, et al. A novel model for predicting intravenous immunoglobulin-resistance in Kawasaki disease: a large cohort study. *Front Cardiovasc Med*. 2023;10:1226592. PMID: 37576105; PMCID: PMC10420135. doi:10.3389/fcvm.2023.1226592
13. Han SB, Lee SY. Macrophage activation syndrome in children with Kawasaki disease: diagnostic and therapeutic approaches. *World J Pediatr*. 2020;16(6):566–574. doi:10.1007/s12519-020-00360-6 Epub 2020 May 16. PMID: 32418074.
14. Wang Y, Huang S, Wang P, et al. Novel predictive scoring system for intravenous immunoglobulin resistance helps timely intervention in Kawasaki disease: the Chinese experience. *J Immunol Res*. 2023;2023:6808323. PMID: 37592925; PMCID: PMC10432039. doi:10.1155/2023/6808323
15. Platnich JM, Muruve DA. NOD-like receptors and inflammasomes: a review of their canonical and non-canonical signaling pathways. *Arch Biochem Biophys*. 2019;670:4–14. doi:10.1016/j.abb.2019.02.008

16. Yu P, Zhang X, Liu N, et al. Pyroptosis: mechanisms and diseases. *Signal Transduct Target Ther.* 2021;6(1):128. doi:10.1038/s41392-021-00507-5 PMID: 33776057; PMCID: PMC8005494.
17. Anzai F, Watanabe S, Kimura H, et al. Crucial role of NLRP3 inflammasome in a murine model of Kawasaki disease. *J Mol Cell Cardiol.* 2020;138:185–196. Epub 2019 Dec 10. PMID: 31836541. doi:10.1016/j.yjmcc.2019.11.158
18. Lee Y, Schulte DJ, Shimada K, et al. Interleukin-1 β is crucial for the induction of coronary artery inflammation in a mouse model of Kawasaki disease. *Circulation.* 2012;125(12):1542–1550. doi:10.1161/CIRCULATIONAHA.111.072769 Epub 2012 Feb 23. PMID: 22361326; PMCID: PMC3337219.
19. Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. *Cell Mol Immunol.* 2021;18(9):2114–2127. doi:10.1038/s41423-021-00740-6 Epub 2021 Jul 28. PMID: 34321623; PMCID: PMC8429580.
20. Huang YH, Lo MH, Cai XY, et al. Epigenetic hypomethylation and upregulation of NLRC4 and NLRP12 in Kawasaki disease. *Oncotarget.* 2018;9(27):18939–18948. doi:10.18632/oncotarget.24851 PMID: 29721174; PMCID: PMC5922368.
21. Shao S, Luo C, Zhou K, et al. Predictive value of serum procalcitonin for both initial and repeated immunoglobulin resistance in Kawasaki disease: a prospective cohort study. *Pediatr Rheumatol Online J.* 2019;17(1):78. doi:10.1186/s12969-019-0379-5 PMID: 31775782; PMCID: PMC6882026.
22. Kobayashi T, Inoue Y, Takeuchi K, et al. Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease. *Circulation.* 2006;113(22):2606–2612. doi:10.1161/CIRCULATIONAHA.105.592865 Epub 2006 May 30. PMID: 16735679.
23. Lim GW, Lee M, Kim HS, et al. Hyponatremia and syndrome of inappropriate antidiuretic hormone secretion in Kawasaki disease. *Korean Circ J.* 2010;40(10):507–513. doi:10.4070/kcj.2010.40.10.507 Epub 2010 Oct 31. PMID: 21088754; PMCID: PMC2978293.
24. Wu S, Long Y, Chen S, et al. A New scoring system for prediction of intravenous immunoglobulin resistance of Kawasaki disease in infants under 1-year old. *Front Pediatr.* 2019;7:514. PMID: 31921727; PMCID: PMC6917618. doi:10.3389/fped.2019.00514
25. Hua W, Sun Y, Wang Y, et al. A new model to predict intravenous immunoglobulin-resistant Kawasaki disease. *Oncotarget.* 2017;8(46):80722–80729. doi:10.18632/oncotarget.21083 PMID: 29113339; PMCID: PMC5655234.
26. Lu Y, Chen T, Wen Y, et al. Prediction of repeated intravenous immunoglobulin resistance in children with Kawasaki disease. *BMC Pediatr.* 2021;21(1):406. doi:10.1186/s12887-021-02876-w PMID: 34530763; PMCID: PMC8444587.
27. Dominguez SR, Martin B, Heizer H, et al. Procalcitonin (PCT) and Kawasaki disease: does PCT correlate with IVIG-resistant disease, admission to the intensive care unit, or development of coronary artery lesions? *J Pediatric Infect Dis Soc.* 2016;5(3):297–302. doi:10.1093/jpids/piv019 Epub 2015 Apr 20. PMID: 26407256.
28. Lee NH, Choi HJ, Kim YH. Clinical usefulness of serum procalcitonin level in distinguishing between Kawasaki disease and other infections in febrile children. *Korean J Pediatr.* 2017;60(4):112–117. doi:10.3345/kjp.2017.60.4.112 Epub 2017 Apr 25. PMID: 28461824; PMCID: PMC5410617.
29. Niu MM, Jiang Q, Ruan JW, et al. Clinical implications of procalcitonin in Kawasaki disease: a useful candidate for differentiating from sepsis and evaluating IVIG responsiveness. *Clin Exp Med.* 2021;21(4):633–643. doi:10.1007/s10238-021-00709-9 Epub 2021 Apr 11. PMID: 33839960; PMCID: PMC8036161.
30. Xie Y, Han B. Exploring the relationship between pyroptosis, infiltrating immune cells and Kawasaki disease with resistance to intravenous immunoglobulin (IVIG) via bioinformatic analysis. *Immunobiology.* 2022;227(5):152261. doi:10.1016/j.imbio.2022.152261 Epub 2022 Aug 17. PMID: 36029669.

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