

Ultrasound Evaluation of Endothelial Dysfunction in Immunoglobulin-Resistant Children with Acute Kawasaki Disease

Yizhou Wen

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Yanfeng Yang (✉ yangfcd@163.com)

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Yang Wen

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Jimei Xi

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Tingting Chen

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Yaheng Lu

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Yiling Liu

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Feifei Si

Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital

Research Article

Keywords: Kawasaki disease, intravenous immune globulin resistance, endothelium dysfunction, ultrasound, cardiology

Posted Date: February 9th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1319360/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Purpose Clinically, some children with Kawasaki disease (KD) who do not respond to the first application of gamma globulin therapy have a longer course of disease and a higher risk of coronary artery damage. The characteristics of vascular endothelial dysfunction in intravenous immune globulin (IVIG)-resistant patients during the acute phase are not well described. We aimed to determine whether indicators that detect the situation of the endothelium are useful parameters that can accurately reflect subclinical dysfunction in resistant patients and may assist in differentiating patients with KD at a higher risk of IVIG resistance.

Methods Fifty IVIG-resistant KD children, 120 IVIG-responsive KD children, 70 febrile children with acute upper respiratory infection and 100 healthy controls were recruited, and indicators reflecting endothelial inflammation, including flow-mediated dilation (FMD), were measured. Receiver operating characteristic (ROC) curve analysis was utilized to determine the threshold values of these parameters associated with IVIG resistance. Multiple logistic regression analysis was performed to determine whether FMD was an independent predictor of IVIG-resistant patients.

Results Compared with normal children, febrile children and IVIG-sensitive KD patients, IVIG-resistant patients had lower FMD ($P < 0.05$) and higher carotid artery intima-media (CIMT) ($P < 0.05$). Laboratory data demonstrated that the percentage of neutrophils (N %) and the levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and serum procalcitonin (PCT) were significantly higher in the IVIG-resistant group than in the IVIG-responsive group, while the levels of albumin (ALB) and serum sodium were significantly lower ($P < 0.05$). PCT, Na⁺ and FMD exhibited AUCs of 0.727, 0.653 and 0.698, respectively, in predicting IVIG resistance in KD through ROC analysis. PCT > 1.69 ng/ml, Na⁺ < 133.2 mmol/l and FMD $< 5.79\%$ were independent predictors of IVIG resistance in KD (OR, 95% CI, $P < 0.05$).

Conclusion More severe endothelial dysfunction, especially lower FMD, was present in IVIG-resistant patients than in IVIG-responsive patients. It is a helpful diagnostic tool that provides supportive criteria to detect KD patients at a higher risk of IVIG resistance when FMD $< 5.79\%$ in children.

What Is Known

Children with Kawasaki disease have declined FMD and Stiffness index (SI) values, as well as raised carotid intima media thickness (CIMT) which are related to the severity of endothelial dysfunction in later stage after the onset.

What is new 1. FMD is lower and CIMT is higher in KD patients with IVIG-resistance than those without, in the acute stage. 2. FMD and PCT have a negative relationship with each other, and FMD $< 5.79\%$ reminds a chance of IVIG-resistance.

Introduction

Kawasaki disease (KD) is a self-limited systemic vasculitis syndrome with acute fever. Although intravenous immunoglobulin (IVIG) in combination with aspirin has been the standard regimen for years[1], approximately 10-20% of children with KD still have persistent fever or repeated typical symptoms of KD 36 hours after the completion of the first IVIG injection, which is called IVIG resistance[2]. Drug-resistant patients are at higher risk of developing coronary artery lesions (CALs) and require additional doses of IVIG and even other interventions, such as corticosteroid infliximab plasma exchange and cytotoxic drugs, which may have potentially adverse consequences and economic costs[3, 4]. The early detection of such resistant patients could greatly help clinicians achieve optimal treatment and prognosis. The physician could timely communicate with the patients' guardians about a doubt on the effectiveness of IVIG before IVIG resistance appears, which would efficiently reduce the medical dispute and then allow choosing personalized therapy according to the degree of IVIG resistance, as estimated using detection indicators.

Several inflammatory laboratory parameters and cytokine markers have been reported to predict IVIG-resistant KD, but they may not be popular in clinical practice because they are invasive, expensive and lack reliability [5-7]. Several scoring systems

proposed by Japanese researchers are also commonly used in the clinical identification of IVIG-resistant patients [8, 9]. However, these systems seem to be difficult to replicate due to a lack of good sensitivity and reproducibility outside Japan [10, 11]. Additionally, vascular ultrasonography parameters are not included in these systems. In fact, as noninvasive ultrasound indicators, flow-mediated dilation (FMD), carotid intima media thickness (CIMT) and stiffness index (SI) have been widely used in recent years in the prognostic follow-up of a cardiovascular risk in chronic diseases such as diabetes, hypertension and metabolic syndrome [12, 13], while an abnormal decrease in FMD and increases in CIMT and SI suggest a subhealthy state of the endothelium. A number of studies have confirmed that children with a history of KD, especially those with coronary artery lesions, maintain these abnormalities from the beginning to the follow-up years of the disease [14, 15]. The pathological basis of KD lies in systemic inflammation of small blood vessels, which means that the immunological mechanism of IVIG resistance is also inextricably linked to endothelial dysfunction. Therefore, it is of great clinical significance to explore the correlation between endothelial function and drug resistance. However, the predictive value of these endothelial indicators in the acute phase of KD has not been fully studied, especially the relation with IVIG resistance. In this study, we measured the endothelial function of IVIG-resistant KD patients and evaluated the predictive value of FMD, CIMT, and SI by comparing them with serum laboratory indicators.

Materials And Methods

Study population

From July 2017 to October 2021, 50 IVIG-resistant and 120 IVIG-responsive patients with KD were randomly recruited in the acute stage of KD at the Chengdu Women's and Children's Central Hospital, School of Medicine, University of Electronic Science and Technology of China using the numbering method in SPSS 20.0. Seventy cases of children with fever due to acute upper respiratory tract infection (AURI) and 100 normal children completing regular check-ups at our hospital, who were matched for age and sex with patients in the KD group, were also randomly enrolled during the same period using the same approach. The diagnosis of complete KD, incomplete KD and coronary artery lesions (CALs) met the diagnostic criteria of the American Heart Association in 2017 [1]. CALs were defined as a dilated coronary artery with a z score of ≥ 2.5 in at least one of the right, left anterior descending, and left main coronary arteries. All KD children were treated with IVIG therapy (2 g/kg over 12 hours). Immunoglobulin resistance was determined as persistent or recurrent fever (under-arm temperature $\geq 37.5^{\circ}\text{C}$) for more than 36 hours but not longer than 7 days after the end of IVIG infusion. The exclusion criteria for all subjects were as follows: (1) previous medical history of KD; (2) previous or current medical history of dyslipidaemia, diabetes, inherited cardiovascular disease, inherited metabolic disease, cystinuria, chronic kidney disease, blood disease, etc.; and (3) any history of major surgery, blood transfusion, or use of vasoactive agents within 3 months before our examination.

Laboratory and clinical data

Laboratory and clinical data were collected through medical record review. Clinical data, such as age, sex, body mass index (BMI), diagnosis of incomplete KD and CALs, and lasting days of fever before first IVIG, were collected. Laboratory data on admission pre-IVIG treatment were collected, including white blood cell count (WBC) percentage of neutrophils (N%), haemoglobin (Hb), platelet (PLT), C-reactive protein (CRP), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum albumin (ALB), serum sodium (Na⁺), procalcitonin (PCT), N-terminal pro-brain natriuretic peptide (NT-proBNP), creatine kinase-MB (CK-MB), D-dimer, and erythrocyte sedimentation rate (ESR).

Assessment of ultrasound data

This series of tests gathered data of all participants, 72 hours after the initial treatment of IVIG and aspirin as well as before other additional treatment for the KD patients. All subjects were asked to sleep late and lay at rest in the supine position for at least 10 min in a quiet room before the ultrasound assessments. Any uncooperative subjects were sedated with chloral hydrate (10% solution at 0.3-0.5 ml/kg, maximum 10 ml). All ultrasonic indicators were measured under standardized conditions by a single experienced sonographer (Wen Y) who was blinded to the diagnoses using an ultrasound machine

(Philips CX50) with a L12-3 transducer, and the average of three repeated test records for each indicator was used for statistical analysis. The KD group underwent additional echocardiography tests and was examined before IVIG treatment.

1. Brachial arterial FMD: FMD was determined with reference to the methods described by Celermajer et al[16] in 1992. The brachial artery was detected above the antecubital fossa of the right arm by the aforementioned ultrasound machine. The distance between the posterior and anterior intima of the vascular wall at the end of diastole (D1) was imaged in the longitudinal axis as a baseline internal diameter. Then, the blood pressure cuff was deflated rapidly after having been inflated to 50 mmHg above the subject's resting systolic blood pressure for 5 minutes to occlude the brachial artery. The diastolic diameter (D2) was recorded sixty seconds later. The FMD value was calculated as $FMD = [(D2 - D1)/D1] \times 100\%$.

2. CIMT: All measurements were performed according to a standardized scanning protocol for the right common carotid arteries[17]. The transducer footprint was manipulated to be parallel to the near and far walls of the common carotid arteries, and the maximum in the longitudinal plane was supposed to be the lumen diameter. The entire carotid proximal common carotid artery was observed approximately 1.5 cm before the bifurcation. The distance between the leading edges of the luminal-intimal interface and the medial-adventitial interface was measured as CIMT.

3. Stiffness index (SI): B-mode ultrasound images were obtained in longitudinal sections 1.5 cm proximal to the right carotid bulb. Carotid artery diameter was measured during systole (Ds) and diastole (Dd). The diastole was coincident with the R wave on the electrocardiogram. A single observer (Xi JM) measured the systolic blood pressure (SBP) and diastolic blood pressure (DBP) three times at intervals of 3 min. The carotid artery wall SI was calculated as $SI = \ln(SBP/DBP) \times (D/\Delta D)$, where $D = (Ds + Dd)/2$ and $\Delta D = Ds - Dd$. Similar methods have been used earlier in previous studies on the subject [18].

4. Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS): The LVEF was acquired by the biplane disk method (modified Simpson's rule)[19], and LVFS was obtained according to the guideline [20].

Statistical analysis

Analyses were performed using SPSS 20.0 software (IBM Co., Armonk, NY, USA). Medians and interquartile ranges were reported for the quantitative ultrasonic and laboratory data. Categorical data are described as counts and percentages. Because some data, such as SI and Na, were not normally distributed, comparisons of continuous variables among groups were evaluated using the Kruskal-Wallis H test, while two groups were compared by the Mann-Whitney U test. Chi-square tests were performed to compare categorical variables. Spearman's partial correlation was used to assess correlations between variables. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the role of different indicators in discriminating IVIG unresponsiveness. The sensitivity and specificity were calculated, and the cut-off value was determined by the Youden index. Multivariate logistic regression was used to identify independent predictors of IVIG resistance in KD. All tests were two-sided, and a value of $P < 0.05$ was considered to indicate statistical significance.

Results

1. Comparison of clinical, ultrasonic and laboratory data among groups. As shown in Table 1, there were no significant differences in baseline characteristics (age, sex, and BMI) or SI among the KD, AURI and normal groups. FMD and CIMT in the KD group were both significantly different from those in the other two groups ($P < 0.01$). Table 2 shows a comparison of the laboratory data between the subgroups of KD. These 2 tables indicate that FMD and the levels of serum Na⁺ and ALB were significantly lower while CIMT, N%, and the levels of serum PCT and NT-proBNP were significantly higher ($P < 0.05$) in the IVIG-resistant group than in the IVIG-responsive group.

2. ROC analysis of different indicators for predicting IVIG resistance. The ROC curves of FMD, CIMT, N%, PCT, ALB, Na⁺ and NT-proBNP for predicting IVIG resistance in children with KD were analysed (Fig. 1; Table 3). In comparison, the sensitivities of FMD were higher than those of other indicators, with a value of 78.0% at its cut-off point of 5.79%. PCT at its cut-off point of 1.69 ng/ml had the largest AUC of 0.727.

Table 1
The clinical and ultrasound data of all participants

Parameter	IR (n= 50)	IS (n= 120)	AURI (n=70)	Normal (n=100)	P
Age** (years)	2.53(1.33~4.12)	2.50(1.60~3.40)	2.78(1.76~3.88)	2.52(1.75~3.61)	0.237
Male**(%)	31(62.0%)	65(54.2%)	40(57.1%)	60(60.0%)	0.846
BMI** (kg/m ²)	16.10(15.33~16.74)	16.05(15.46~16.74)	15.88(15.00~16.73)	16.11(15.34~16.71)	0.605
iKD(%)	7(14.0%)	14(11.7%)	-	-	0.798
CAL(%)	13(26.0%)	29(24.2%)	-	-	0.846
FMD(%)	4.03(2.26~5.74) ^{a,b,c}	7.25(4.42~9.42) ^{a,b}	12.76(10.05~15.59)	14.18(11.17~16.71)	<0.001*
SI**	2.34(1.96~2.72)	2.26(2.01~2.68)	2.25(1.98~2.67)	2.24(2.00~2.64)	0.758
CIMT(mm)	0.387(0.326~0.455) ^{a,b,c}	0.356(0.310~0.413) ^a	0.339(0.294~0.413)	0.331(0.283~0.395)	<0.001*
LVEF(%)	65.0(61.7~69.9)	65.8(62.5~68.4)	-	-	0.484
LVFS(%)	36.3(32.5~38.0)	38.2(32.8~40.5)	-	-	0.091
<p>IR,IVIG-resistant; IS,IVIG-sensitive; AURI, acute upper respiratory infection; KD, Kawasaki disease; BMI, body mass index; iKD incomplete Kawasaki disease; CAL coronary artery lesion; IVIG, intravenous immunoglobulin; FMD, brachial artery flow-mediated dilation; SI, stiffness index; CIMT, carotid intima media thickness; LVEF, left ventricular ejection fraction. LVFS, left ventricular fractional shortening. ^aP < 0.05, compared with the Normal group; ^bP < 0.05, compared with the AURI group; ^cP < 0.05, compared with the IS group;—, this data was not detected; **P > 0.05(IR v. s. AURI, IR v. s. Normal, IR v. s. IS, IS v. s. Normal, AURI v. s. Normal); *Statistically significant (P< 0.05).</p>					

Table 2
Comparison of laboratory data between the IVIG-resistant group and IVIG-sensitive group

Parameter	IR group (n= 50)	IS group (n= 120)	P
WBC (×10 ⁹ /L)	14.89(11.14~17.84)	14.17(11.93~17.20)	0.457
N%	76.34(70.17~83.27)	71.47(60.01~79.60)	0.01*
HGB(g/L)	111(103~116)	114(107~121)	0.061
PLT(×10 ⁹ /L)	292(213~332)	297(251~376)	0.071
CRP(mg/L)	96.9(73.2~126.4)	86.2(51.5~119.2)	0.153
PCT(ng/ml)	1.87(0.62~3.31)	0.56(0.17~1.22)	<0.001*
ALT(U/L)	37.7(27.2~45.6)	34.7(24.4~42.2)	0.212
AST(U/L)	35.0(28.8~41.5)	32.1(25.9~39.2)	0.114
ALB(g/L)	33.8(30.5~36.8)	36.4(34.1~38.7)	0.001*
Na+(mmol/L)	135.1(132.4~137.0)	136.7(135.1~138.3)	0.002*
NT-proBNP(pg/ml)	649.5(217.8~936.5)	442.9(173.4~756.6)	0.027*
CK-MB(U/L)	1.5(1.0~1.9)	1.4(1.1~1.7)	0.339
D-dimer(ug/ml)	1.17(0.85~2.18)	0.94(0.58~1.84)	0.065
ESR(mm/h)	74.2(51.1~90.4)	71.0(49.7~87.0)	0.321
IR, IVIG-resistant; IS, IVIG-sensitive; WBC, white blood cell; N%, percentage of neutrophils; HGB, hemoglobin; PLT, platelet; CRP, C-reactive protein; PCT, procalcitonin; ALT, alanine amino transferase; AST, aspartate transaminase; ALB, albumin; Na+, serum sodium; NT-proBNP, N-terminal pro-brain natriuretic peptide; CK-MB, creatine kinase-MB; ESR, erythrocyte sedimentation rate. *Statistically significant (P< 0.05)			

Table 3
ROC curve analysis of indicators to predict IVIG resistance

Indicator	AUC	P	95% CI	Cut-off	Sen (%)	Spe (%)	YI
FMD	0.698	<0.001	0.606~0.791	5.79%	78.0	68.3	0.463
CIMT	0.597	0.047	0.500~0.694	0.379mm	56.0	64.2	0.202
N%	0.625	0.010	0.537~0.713	72.16%	74.0	51.7	0.257
PCT	0.727	<0.001	0.632~0.822	1.69ng/ml	56.0	86.7	0.427
ALB	0.669	0.001	0.579~0.759	33.6g/L	50.0	79.2	0.292
Na+	0.653	0.002	0.559~0.747	133.2mmol/L	40.0	89.2	0.292
NT-proBNP	0.608	0.027	0.515~0.701	703.4pg/ml	48.0	70.8	0.188
Sen, Sensitivity; Spe, Specificity; YI, Youden index; FMD, brachial artery flow-mediated dilation; CIMT, carotid intima media thickness; N%, percentage of neutrophils; Na+, serum sodium; ALB, albumin; PCT, procalcitonin; NT-proBNP, N-terminal pro-brain natriuretic peptide; AUC, area under curve.							

3. Logistic regression analysis of IVIG resistance in Kawasaki disease. Using FMD< 5.79%, CIMT>0.379 mm, N%>72.16%, PCT>1.69 ng/ml, ALB>33.6 g/L, Na+<133.2 mmol/L and NT-proBNP>703.4 pg/ml as binary independent variables, multivariate logistic regression for IVIG resistance during the acute phase of KD was analysed and is presented in Table 4. The results showed that FMD< 5.79% (P=0.014), PCT>1.69 ng/ml (P = 0.005) and Na+<133.2 mmol/L (P=0.015) were independent predictors of IVIG resistance in KD children.

4. Correlation among the independent predictors in different groups. Spearman's partial correlation (Fig. 2) showed that there were strong negative correlations between FMD and PCT in all groups, such as the IVIG-resistant group (r= -0.630, p<0.01), IVIG-sensitive group (r= -0.477, p<0.01) and KD group (r= -0.608, p<0.01), while there was a weak positive correlation between FMD and Na+ only in the KD group (r= 0.212, P=0.005).

Table 4
Multivariate logistic regression model composing indicators for predicting IVIG resistance

Indicator	P	OR	95%CI
FMD	0.014	3.563	1.299~9.772
CIMT	0.093	2.069	0.885~4.836
N%	0.353	1.552	0.614~3.924
PCT	0.005	4.257	1.549~11.700
ALB	0.113	2.054	0.843~5.005
Na+	0.015	3.516	1.277~9.680
NT-proBNP	0.137	0.471	0.174~1.270

FMD, brachial artery flow-mediated dilation; CIMT, carotid intima media thickness; N%, percentage of neutrophils; Na+, serum sodium; ALB, albumin; PCT, procalcitonin; NT-proBNP, N-terminal pro-brain natriuretic peptide;

Discussion

There is increasing evidence that the current mechanism of IVIG in the treatment of KD mainly involves the immune response, the destruction and remodelling of the blood vessels and endothelial inflammation, including regulating the activation of T cells and other antigen-presenting cells; regulating Fc receptor expression and inhibiting complement activation; regulating the production of cytokines and inhibiting immune response; neutralizing bacterial super antigens and toxins; and so on [21-23]. Based on these perspectives, it is hypothesized that laboratory markers of inflammation and endothelial function parameters would be reliable predictors. There have been numerous studies [24-26] about the predictive value of laboratory markers for IVIG resistance in KD, but few reports have examined endothelial function parameters and the correlation between these two main indicator types.

In our investigation, we demonstrated that IVIG-resistant KD patients had both significantly lower FMD and higher CIMT than other groups, despite the differences in SI in these groups not reaching statistical significance. However, we did not find any difference in endothelial parameters between AURI patients and normal children. These findings confirmed that common infection-related febrile factors may not be sufficient to cause either obvious swelling of the vascular medial membrane or serious damage to the structure of the arterial wall and that simple and transient inflammation in the acute phase may not be sufficient to cause significant premature arteriosclerosis in a short time [27]. In addition, there was lower FMD and higher CIMT in IVIG-resistant patients than in the IVIG-sensitive patients, which means that endothelial dysfunction in IVIG-resistant KD patients may be more serious than that in IVIG-sensitive patients and AURI patients. There was no significant difference in CIMT between the IVIG-sensitive and AURI groups. Therefore, FMD may be more reliable in KD children for the assessment of endothelial function in the acute phase than CIMT and SI. In further logistic analysis, we found that of the three vascular

ultrasonography parameters, only FMD (< 5.79%) was an independent predictor of IVIG resistance, which also confirmed the above hypothesis. FMD was created as a high-frequency ultrasound technique for a surrogate marker of endothelial vasodilation function by Celermajer et al [16] in 1992. It works by physically blocking the brachial artery, resulting in transient ischaemia and hypoxia, which stimulates the endothelium to release nitric oxide and other associated vasodilators (neurohormones such as acetylcholine, 5-hydroxytryptamine and catecholamine). When the physical blockage is removed, vessels with normal endothelial function expand in response to a large number of these cytokines and molecules [28]. Compared with SI and CIMT, which focus on evaluating vascular morphology [29], FMD mainly monitors vasodilation function after artificial mechanical stimulation physiologically [16, 30]. Since endothelial cell activation and dysfunction are earlier observable changes than structure and form in the development of vascular diseases in lesion areas[31] and measurement of FMD includes online monitoring of disturbed blood flow dynamics, FMD can more sensitively reflect the difference between the vascular endothelium of patients with IVIG resistance and other groups under the influence of systemic inflammation. In adults, FMD<5 indicates impaired endothelial function, but there is no established standard in children [32]. In the present study, the cut-off value predicted by FMD for IVIG resistance was lower than the normal range but significantly lower than that of the healthy control group. Therefore, it was speculated that most IVIG-resistant KD patients had subclinical endothelial dysfunction, as the correlation between them may be undesirable consequences of inflammatory storms.

In the analysis of laboratory markers of inflammation in KD patients, similar to the distinctly different vascular ultrasonography parameters between the IVIG-sensitive and IVIG-resistant groups mentioned above, there were statistically significant differences in NT-proBNP, which reflects myocardial ischaemia and hypoxia; Na⁺, which mainly reflects microvascular permeability and interleukin levels; and inflammatory parameters, such as N%, PCT and ALB, which also support the release and cascade reaction of systemic inflammatory factors and vasculitis in IVIG-resistant patients, which were stronger and more inadequate for remission, consistent with some results of previous studies[24-26]. Among those laboratory data, aggressive inflammatory response (PCT>1.69 ng/ml) and hyponatremia (Na⁺<133.2 mmol/L) were found to be strong independent predictors of IVIG resistance in KD children, similar to FMD (<5.79%), through multivariate logistic regression analysis. Only PCT had a larger AUC than FMD, which had the highest sensitivity with a value of 78.0%, indicating that FMD could be a better predictor than most of the laboratory data above. PCT is an acute-phase reactant whose levels are not affected by the administration of corticosteroids or nonsteroidal anti-inflammatory medication. As a prohormone for calcitonin, it is suppressed or nonresponsive to interferon- γ , moderately responsive to tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) stimulation, and strongly responsive to interleukin-1 β (IL-1 β) [26]. Previous studies reported that increased IL-6 and IL-1 β may activate ADH secretion and aggravate microvascular permeability in KD patients with severe inflammation, leading to SIADH and hyponatremia[25]. This process partially has the same inflammatory mediators as PCT, which means that PCT is partly correlated with microvascular permeability. Both hyponatremia and elevated PCT are closely related to excessive expression of inflammatory factors.

In the context of immune-mediated injury, the vascular endothelium may trigger a low-level inflammatory response by imitating pathogen- and damage-associated molecular patterns and activating monocyte-derived macrophages, T cells, B cells, dendritic cells and mast cells by affecting substrate-receptor interactions and the secretion of different mediators[30] in inflammation, coagulation, angiogenesis and tumour invasion. In our follow-up correlation analysis, the negative correlations between FMD and PCT in both the KD group and subgroups were stronger in the IR group than in the IS group, while hyponatremia was not significantly correlated with FMD since a possibly larger part of FMD involves endocrine and neuroregulatory mechanisms[25] rather than endothelial inflammation. These findings further confirmed that a severe exaggerated inflammatory response can lead to vascular damage through biochemical and immune damage pathways[30] and is an important mechanism of vascular structure and endothelial function damage in IVIG-resistant patients[33, 34]. At the same time, there may be considerable overlap between inflammatory responses that lead to elevated PCT and pathophysiological processes (including injury of endothelial cells that release vasodilators and blocking of vasodilator response pathways in the vascular wall that influence vasodilation) in IVIG-resistant patients. This may be related to cytokines such as TNF- α , IL-6, and IL-1 β [30]. Even though FMD<5.79% (OR=3.563) has a good predictive ability for IR,

PCT>1.69 ng/ml had a higher OR (4.257), which means that abnormally elevated PCT has a stronger directivity to IR than FMD. Therefore, we speculate that changes in the morphology and function of vessel walls lag behind inflammation in KD[35], which is also believed to be the initial step in many other rheumatic diseases, including systemic lupus erythematosus, ankylosing spondylitis, Behcet's disease, rheumatoid arthritis, and so on[36].

Although further basic experimental proof is needed, the above conclusions might provide a clue for future aetiological studies on IVIG resistance in KD. Additionally, we suggest that before IVIG treatment, if either FMD, PCT or Na is found to be abnormally different from their critical values, we recommend the timely strengthening of endothelial nutritional support, extra anti-inflammatory therapy such as hormone application, regulation of water and electrolyte homeostasis and other measures that can promote the control of inflammation to a certain extent to prevent the occurrence of IVIG resistance. Similarly, in children with drug resistance to IVIG, strengthening the above measures is likely to prevent the recurrence of drug resistance and refractory KD [3, 23, 37, 38]. While the combination of FMD and PCT as a diagnostic tool in routine clinical practice is not yet warranted, in the absence of reliable tools for diagnosing IVIG-resistant KD, this investigation could prove useful in the future.

Our study has several limitations. First, the sample size of this study was small, and all subjects in this study were Chinese. Further large-scale, multicentre prospective studies are needed to confirm our findings. Second, to acquire ultrasonic data, we measured only the right neck for CIMT and SI and only the left brachial artery for FMD, while LVEF and LVFS were measured only in the KD group, which may have led to some selection bias. Third, the laboratory data were limited in our study; we did not collect laboratory data for the AURI and normal groups, and thus, more indicators need to be included prospectively.

Conclusion

IVIG-resistant KD patients have more severe endothelial dysfunction than IVIG-sensitive patients. Different methods of measuring the endothelium with high-frequency ultrasound probes are effective tools for detecting subclinical endothelial dysfunction in IVIG-resistant patients. FMD may be a more useful diagnostic parameter than CIMT and SI and may provide a supporting standard for the detection of KD patients in the acute stage with a higher risk of IVIG resistance.

Abbreviations

ALB serum albumin

ALT serum alanine aminotransferase

AST serum aspartate aminotransferase

AUC Area under the curve

AURI acute upper respiratory tract infection

BMI body mass index

CALs Coronary artery lesions

CIMT carotid intima media thickness

CK-MB creatine kinase-MB

CRP C-reactive protein

DBP diastolic blood pressure

ESR and erythrocyte sedimentation rate

FMD Flow-mediated dilatation

IVIG intravenous immunoglobulin

KD Kawasaki disease

LVEF Left ventricular ejection fraction

LVFS left ventricular fractional shortening

Na⁺ serum sodium

NT-proBNP N-terminal pro-brain natriuretic peptide

PCT procalcitonin

ROC Receiver operating characteristic

SI stiffness index

SBP systolic blood pressure

WBC white blood cell count

N% percentage of neutrophils

Hb haemoglobin

PLT platelet

Declarations

Funding: This study was funded by the Scientific Research Fund of Sichuan Health Commission (No. 17PJ269) and Scientific Research Fund of Chengdu Health Commission (No. 2021131). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and material: The original contributions presented in the study are included in the article supplementary material, further inquiries can be directed to the corresponding authors.

Data availability: All data was stored following our Ethics Committee and data files are kept in our Hospitals Servers and are available for further analysis and data transparency.

Code availability: Statistical analysis was done with SPSS v 20.0, and syntax files are available with syntax's code fully auditable.

Authors' Contributions: (I) Conception and design: WYZ, YY; (II) Administrative support: YY, CT; (III) Provision of study materials or patients: WYZ, YY; (IV) Collection and assembly of data: WYZ, XJ, WY and LY; (V) Data analysis and interpretation: YY, LY and SF; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Ethical approval: This study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and was approved by the Ethics Committee of the Chengdu Women's and Children's Central Hospital (approval number: 2017(11)). Clinical informed written consent was obtained from the guardians of each child.

Consent to participate: Consent is not required as all the data is anonymized, and the submission does not include images that may identify.

Consent for publication: Consent is not required as all the data is anonymized, and the submission does not include images that may identify the person.

References

1. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, Baker AL, Jackson MA, Takahashi M, Shah PB *et al*: **Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association.** *Circulation* 2017, **135**(17):e927-e999.
2. Duignan S, Doyle SL, McMahon CJ: **Refractory Kawasaki disease: diagnostic and management challenges.** *Pediatric Health Med Ther* 2019, **10**:131-139.
3. Zhang RL, Lo HH, Lei C, Ip N, Chen J, Law BY: **Current pharmacological intervention and development of targeting IVIG resistance in Kawasaki disease.** *Curr Opin Pharmacol* 2020, **54**:72-81.
4. Portman MA, Dahdah NS, Slee A, Olson AK, Choueiter NF, Soriano BD, Buddhe S, Altman CA, Investigators E: **Etanercept With IVIg for Acute Kawasaki Disease: A Randomized Controlled Trial.** *Pediatrics* 2019, **143**(6).
5. Liu X, Shao S, Wang L, Zhang N, Wu M, Liu L, Zhao L, Hua Y, Zhou K, Luo C *et al*: **Predictive Value of the Systemic Immune-Inflammation Index for Intravenous Immunoglobulin Resistance and Cardiovascular Complications in Kawasaki Disease.** *Front Cardiovasc Med* 2021, **8**:711007.
6. Kitoh T, Ohara T, Muto T, Okumura A, Baba R, Koizumi Y, Yamagishi Y, Mikamo H, Daigo K, Hamakubo T: **Increased Pentraxin 3 Levels Correlate With IVIG Responsiveness and Coronary Artery Aneurysm Formation in Kawasaki Disease.** *Front Immunol* 2021, **12**:624802.
7. Wang Y, Qian SY, Yuan Y, Wang Q, Gao L, Chen X, Yu X, Zhen Z: **Do cytokines correlate with refractory Kawasaki disease in children?** *Clin Chim Acta* 2020, **506**:222-227.
8. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, Kobayashi T, Morikawa A: **Prediction of intravenous immunoglobulin unresponsiveness in patients with Kawasaki disease.** *Circulation* 2006, **113**(22):2606-2612.
9. Egami K, Muta H, Ishii M, Suda K, Sugahara Y, Iemura M, Matsuishi T: **Prediction of resistance to intravenous immunoglobulin treatment in patients with Kawasaki disease.** *J Pediatr* 2006, **149**(2):237-240.
10. Sleeper LA, Minich LL, McCrindle BM, Li JS, Mason W, Colan SD, Atz AM, Printz BF, Baker A, Vetter VL *et al*: **Evaluation of Kawasaki disease risk-scoring systems for intravenous immunoglobulin resistance.** *J Pediatr* 2011, **158**(5):831-835 e833.
11. Song R, Yao W, Li X: **Efficacy of Four Scoring Systems in Predicting Intravenous Immunoglobulin Resistance in Children with Kawasaki Disease in a Children's Hospital in Beijing, North China.** *J Pediatr* 2017, **184**:120-124.
12. Donald AE, Charakida M, Cole TJ, Friberg P, Chowienzyk PJ, Millasseau SC, Deanfield JE, Halcox JP: **Non-invasive assessment of endothelial function: which technique?** *J Am Coll Cardiol* 2006, **48**(9):1846-1850.
13. Sitia S, Tomasoni L, Atzeni F, Ambrosio G, Cordiano C, Catapano A, Tramontana S, Perticone F, Naccarato P, Camici P *et al*: **From endothelial dysfunction to atherosclerosis.** *Autoimmun Rev* 2010, **9**(12):830-834.
14. Dhillon R, Clarkson P, Donald AE, Powe AJ, Nash M, Novelli V, Dillon MJ, Deanfield JE: **Endothelial dysfunction late after Kawasaki disease.** *Circulation* 1996, **94**(9):2103-2106.
15. Dietz SM, Tacke CE, Hutten BA, Kuijpers TW: **Peripheral Endothelial (Dys)Function, Arterial Stiffness and Carotid Intima-Media Thickness in Patients after Kawasaki Disease: A Systematic Review and Meta-Analyses.** *PLoS One* 2015,

16. Celermajer DS SK, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE: **Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis.** *Lancet* 1992, Nov 7;340(8828):1111-5.
17. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS, American Society of Echocardiography Carotid Intima-Media Thickness Task F: **Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine.** *J Am Soc Echocardiogr* 2008, **21**(2):93-111; quiz 189-190.
18. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, Jacobson M, Mahoney L, Mietus-Snyder M, Rocchini A *et al*: **Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association.** *Hypertension* 2009, **54**(5):919-950.
19. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS *et al*: **Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology.** *J Am Soc Echocardiogr* 2005, **18**(12):1440-1463.
20. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, Lai WW, Geva T: **Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council.** *J Am Soc Echocardiogr* 2010, **23**(5):465-495; quiz 576-467.
21. Leung DY: **The immunologic effects of IVIG in Kawasaki disease.** *Int Rev Immunol* 1989, **5**(2):197-202.
22. Chang L, Yang HW, Lin TY, Yang KD: **Perspective of Immunopathogenesis and Immunotherapies for Kawasaki Disease.** *Front Pediatr* 2021, **9**:697632.
23. He M, Chen Z, Martin M, Zhang J, Sangwung P, Woo B, Tremoulet AH, Shimizu C, Jain MK, Burns JC *et al*: **miR-483 Targeting of CTGF Suppresses Endothelial-to-Mesenchymal Transition: Therapeutic Implications in Kawasaki Disease.** *Circ Res* 2017, **120**(2):354-365.
24. Wu S, Liao Y, Sun Y, Zhang CY, Zhang QY, Yan H, Qi JG, Liu XQ, Chen YH, Wang YL *et al*: **Prediction of intravenous immunoglobulin resistance in Kawasaki disease in children.** *World J Pediatr* 2020, **16**(6):607-613.
25. Lim GW, Lee M, Kim HS, Hong YM, Sohn S: **Hyponatremia and syndrome of inappropriate antidiuretic hormone secretion in kawasaki disease.** *Korean Circ J* 2010, **40**(10):507-513.
26. Dominguez SR, Martin B, Heizer H, Jone PN, Tong S, Davidson J, Anderson MS, Glode MP: **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.
27. Mori Y, Katayama H, Kishi K, Ozaki N, Shimizu T, Tamai H: **Persistent high fever for more than 10 days during acute phase is a risk factor for endothelial dysfunction in children with a history of Kawasaki disease.** *J Cardiol* 2016, **68**(1):71-75.
28. Cahill PA, Redmond EM: **Vascular endothelium - Gatekeeper of vessel health.** *Atherosclerosis* 2016, **248**:97-109.
29. Calabro MP, Carerj S, Russo MS, Luca FL, Onofrio MT, Antonini-Canterin F, Zito C, Oreto L, Manuri L, Khandheria BK *et al*: **Carotid artery intima-media thickness and stiffness index beta changes in normal children: role of age, height and sex.** *J Cardiovasc Med (Hagerstown)* 2017, **18**(1):19-27.
30. Balta S: **Endothelial Dysfunction and Inflammatory Markers of Vascular Disease.** *Curr Vasc Pharmacol* 2021, **19**(3):243-249.
31. Theodorou K, Boon RA: **Endothelial Cell Metabolism in Atherosclerosis.** *Front Cell Dev Biol* 2018, **6**:82.

32. Hopkins ND, Dengel DR, Stratton G, Kelly AS, Steinberger J, Zavala H, Marlatt K, Perry D, Naylor LH, Green DJ: **Age and sex relationship with flow-mediated dilation in healthy children and adolescents.** *J Appl Physiol (1985)* 2015, **119**(8):926-933.
33. Galeotti C, Kaveri SV, Cimaz R, Kone-Paut I, Bayry J: **Predisposing factors, pathogenesis and therapeutic intervention of Kawasaki disease.** *Drug Discov Today* 2016, **21**(11):1850-1857.
34. Napoli R, Ruvolo A, Triggianese P, Prevete N, Schiattarella GG, Nigro C, Miele C, Magliulo F, Grassi S, Pecoraro A *et al*: **Immunoglobulins G modulate endothelial function and affect insulin sensitivity in humans.** *Nutr Metab Cardiovasc Dis* 2020, **30**(11):2085-2092.
35. Dalla Pozza R, Bechtold S, Urschel S, Kozlik-Feldmann R, Netz H: **Subclinical atherosclerosis, but normal autonomic function after Kawasaki disease.** *J Pediatr* 2007, **151**(3):239-243.
36. Wu TH, Kuo HC, Tain YL, Lin KM, Kuo HC, Chien SJ: **Common carotid artery intima-media thickness is useful for diagnosis of the acute stage of Kawasaki disease.** *BMC Pediatr* 2014, **14**:98.
37. Akamine K, Punaro M: **Biologics for childhood systemic vasculitis.** *Pediatr Nephrol* 2019, **34**(11):2295-2309.
38. Medina-Leyte DJ, Zepeda-Garcia O, Dominguez-Perez M, Gonzalez-Garrido A, Villarreal-Molina T, Jacobo-Albavera L: **Endothelial Dysfunction, Inflammation and Coronary Artery Disease: Potential Biomarkers and Promising Therapeutical Approaches.** *Int J Mol Sci* 2021, **22**(8).

Figures

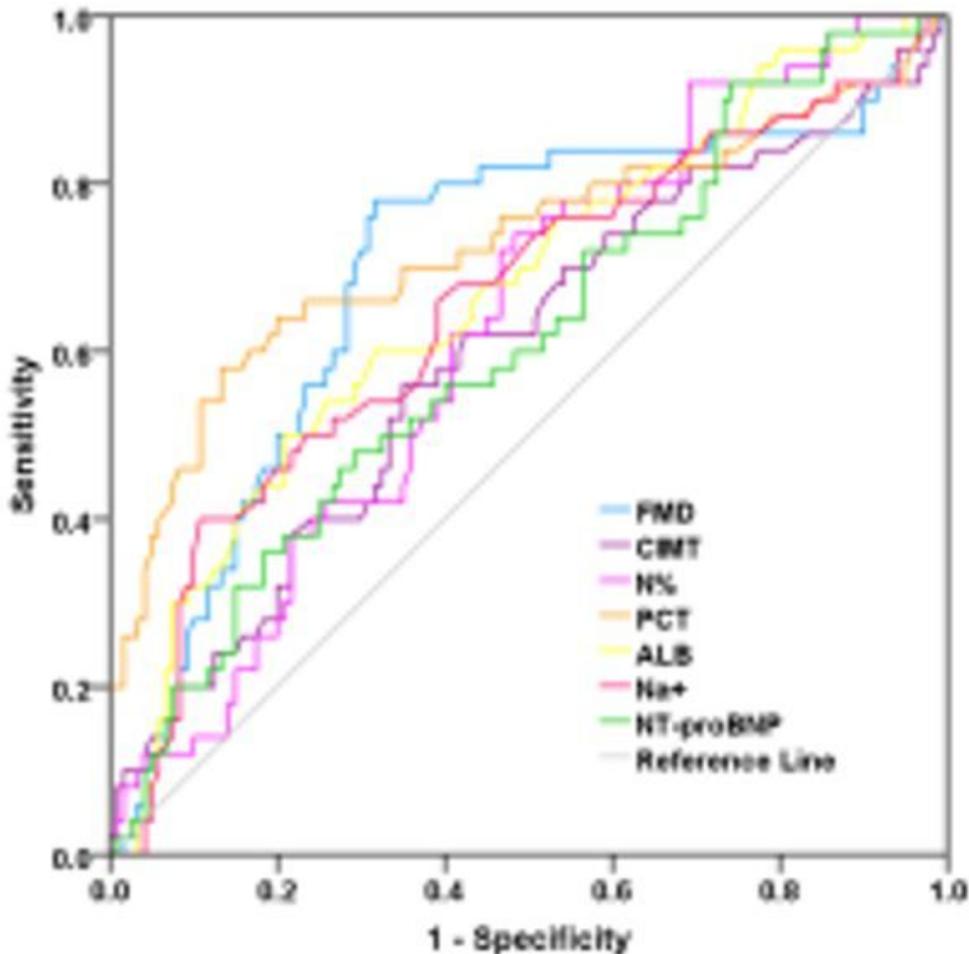


Figure 1

ROC curve analysis of indicators in predicting IVIG resistance

FMD, brachial artery flow-mediated dilation; CIMT, carotid intima media thickness; N% percentage of neutrophils; Na⁺, serum sodium; ALB, albumin; PCT, procalcitonin; NT-proBNP, N-terminal pro-brain natriuretic peptide.

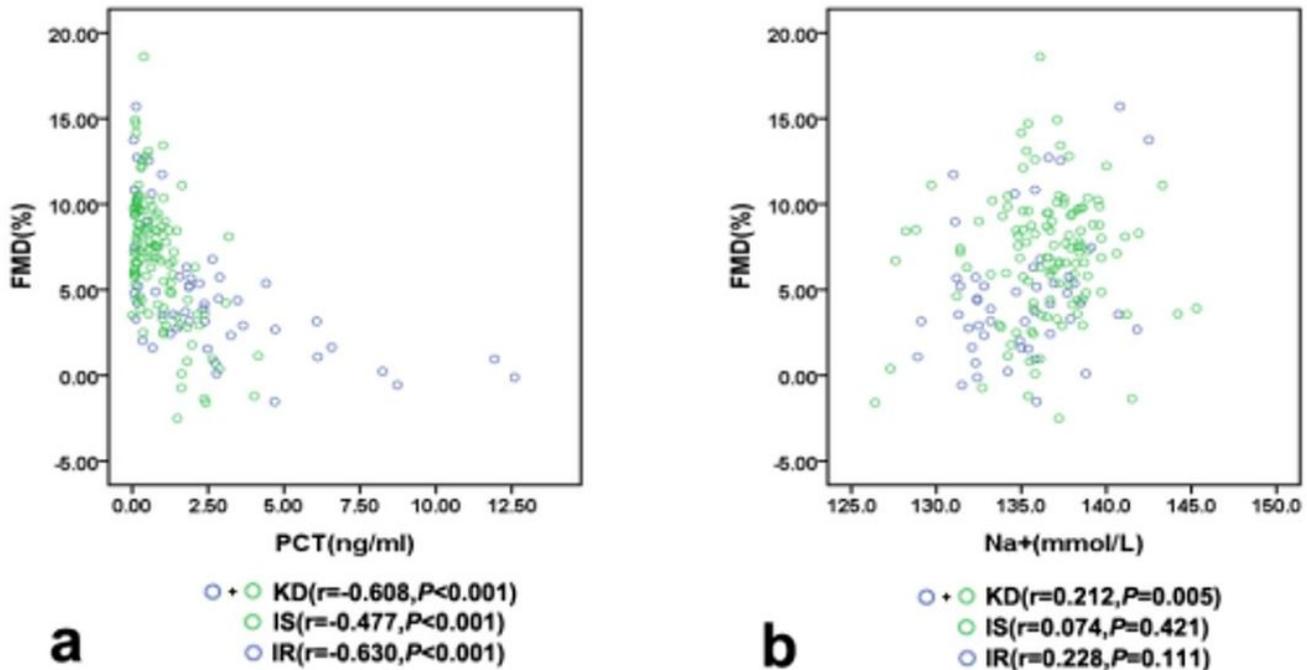


Figure 2

Correlations between flow-mediated dilation (FMD) and two serum indicators (sodium and procalcitonin) in three different groups (KD group, IVIG-resistant group and IVIG-sensitive group)

KD, Kawasaki disease; IS, IVIG-sensitive; IR, IVIG-resistant. Scatter plots showing (a) correlation between FMD and procalcitonin in the KD, IS and IR groups; (b) correlation between FMD and sodium in the KD, IS and IR groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [1.png](#)
- [2.png](#)
- [3.png](#)
- [4.png](#)