

# Impact factors for refractory KD and comparison of different treatment protocols

**Ling Wu**

Ningbo Women and Children's Hospital

**Zhengjie Chen**

Ningbo University

**Shiling Zhong**

Ningbo Women and Children's Hospital

**Fangfang Hu**

Ningbo university

**Yazhen Di** (✉ [dyz028sci@163.com](mailto:dyz028sci@163.com))

Ningbo Women and Children's Hospital

---

## Research article

**Keywords:** Coronary artery lesion (CAL), glucocorticoid (GC), intravenous immunoglobulin (IVIG), Kawasaki disease (KD), refractory type

**Posted Date:** June 2nd, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-32200/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

## Background

Intravenous immunoglobulin (IVIG) has been widely accepted as a standard treatment against Kawasaki disease (KD). About 20% of patients are nonresponsive after the first IVIG treatment. This study was performed to reveal the responsiveness, mechanism of action, and incidence rate of coronary artery lesions in the long-term follow-up visit of action of IVIG combined with glucocorticoids in the treatment of refractory KD.

## Method:

A total of 633 pediatric patients with KD (experimental group) and 200 pediatric patients with upper respiratory infection hospitalized during the same period (control group) were retrospectively selected. KD was treated with IVIG combined with aspirin. IVIG-nonresponsive group was given the second treatment. Protocol 1 group was administered 1 g/kg IVIG; Protocol 2 group was administered 2 g/kg IVIG; and Protocol 3 group was administered 1 g/kg IVIG + glucocorticoid (GC). Following the second treatment, the responsive group (Group A) and nonresponsive group (Group B) were classified based on whether the temperature was reduced to normal.

## Results

IVIG-nonresponsive group had more coronary artery lesions, longer time of first application of IVIG, and higher WBC count, ESR, CRP, and higher serum levels of Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10. Protocol 3 group was found with faster abatement of fever, higher response rate, and fewer CALs. The serum levels of Meprin A, IL-1 $\beta$ , and IL-17A in Group B were higher, and the incidence rate of CAL after treatment was up to 78.9% .

## Conclusions

The levels of Meprin A, IL-17A, and IL-1 $\beta$  may serve as potentially good indexes to predict refractory KD. IVIG combined with GC to treat refractory KD is more advantageous compared with IVIG alone.

## Background

Kawasaki disease (KD) is a kind of pediatric systemic vasculitis of unknown etiology. Intravenous immunoglobulin (IVIG) has been widely accepted as a standard treatment against KD. About 20% of patients are nonresponsive after the first IVIG treatment, also referred to as "refractory KD." Pediatric patients with this condition are more exposed to coronary artery lesions (CALs) [1]. Clinical treatment protocols available for these pediatric patients with KD include high-dose glucocorticoid (GC) treatment,

second IVIG treatment, ulinastatin (UTI) treatment, methotrexate (MTX) treatment, cyclosporin treatment, infliximab (IFX) treatment, and plasmapheresis [2, 3]. However, no agreement has been reached on the preferred second-line drugs for such pediatric patients in the community. In US clinical practice, about 7.7–18% of pediatric patients with KD nonresponsive to the first IVIG treatment were given GC [4]. In another Spanish KD cohort study, about 14.5% of pediatric patients with KD were given GC in the first or second treatment [4]. In China, GC was also used in the first and second treatment of KD [2, 5]. Hence, the treatment outcome of GC in KD has aroused immense attention at home and abroad. However, its responsiveness, mechanism of action, and incidence rate of CAL in the long-term follow-up visit remain unclear. This study was performed to reveal the application value and mechanism of action of IVIG combined with glucocorticoids in the treatment of refractory KD.

## Participants And Methods

### Participants

This retrospective study included 633 pediatric patients with KD who underwent standard IVIG treatment in the hospital from January 2016 to March 2019. The inclusion criterion was pediatric patients with KD having a definitive diagnosis and complete data, referring to the diagnostic criteria proposed by American Heart Association 2017 [6]. Patients complicated with other autoimmune diseases, infectious diseases, and tumors were excluded. Further, 200 pediatric patients with an upper respiratory infection and hospitalized in the hospital during the same period were considered as the control group. Two groups of pediatric patients were not given hormones, immunosuppressants, or IVIG within 2 weeks. The experimental group received standard IVIG treatment combined with aspirin and was subdivided into IVIG-responsive group and IVIG-nonresponsive group based on the response to IVIG treatment (IVIG nonresponsive was defined as persistent fever of  $>38^{\circ}\text{C}$  for 36 h after the first-dose IVIG treatment, abatement of fever for 2–7 days after drug administration, fever recurring after 2 weeks, with at least one of KD symptoms). The IVIG-nonresponsive group received the second treatment and then was distributed into Protocol 1 group (20 participants), Protocol 2 group (36 participants), and Protocol 3 group (30 participants). Following the second treatment, the IVIG-nonresponsive group was further classified into responsive group (Group A) and nonresponsive group (Group B), depending on whether the fever was abated. The family members of all pediatric patients included in this study signed the informed consent, and the approval was obtained from the Medical Ethics Committee of the hospital under the grant number: [2018] Lun Shen Zi (22).

### Methods

General data: The clinical and experimental data of all the pediatric patients with KD during hospitalization and follow-up visit were recorded. The blood drawn from the experimental and control groups when admitted to the hospital was retained for testing the serum levels of Meprin A, interleukin-1 $\beta$  (IL-1 $\beta$ ), and interferon-induced protein-10 (IP-10). White blood cell (WBC) count, erythrocyte sedimentation

rate (ESR), and levels of C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-17A (IL-17A) were tested during hospitalization. The time of fever abatement (the time from the day of second treatment to the resumption of normal body temperature without a recurrent fever) and the experimental data before and after the second treatment (pretreatment: prior to the second IVIG treatment, i.e., within 3–5 days after the first IVIG treatment; post-treatment: within 3–5 days after the second IVIG treatment), including WBC count, ESR, and levels of CRP, Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10, were recorded for pediatric patients with KD in the IVIG-nonresponsive group. All the pediatric patients with KD in these groups were subjected to an ultrasonic cardiogram test from the onset to the 1-year follow-up visit (1, 2, 4, 8, and 12 weeks, and 6 and 12 months after the onset). The findings suggested coronary artery dilation or coronary aneurysm, defined as CAL. The diagnostic criterion for coronary artery dilation was as follows: coronary artery diameter >3 mm if participants aged <5 years; and coronary artery diameter >4 mm if participants aged >5 years. The diagnostic criterion for coronary artery aneurysm was as follows: coronary artery localized or diffused dilation, and its diameter 1.5 times higher compared with the adjacent normal coronary artery. Small coronary aneurysm was identified if the inner diameter was 4.1–4.9 mm; medium coronary aneurysm was identified if the inner diameter was 5.0–7.9 mm; and huge coronary aneurysm was identified if the inner diameter was  $\geq$ 8 mm. Post-treatment CALs included CALs appearing during the follow-up visit after the second treatment, excluding those found before the second treatment.

For serum meprin A, IL-1 $\beta$ , and IP-10 test, 4 mL of fasting venous blood was drawn from all pediatric patients with KD during the acute phase (course of disease: 3–7 days), 4 mL from the IVIG-nonresponsive group within 3–5 days after the first IVIG treatment, and 4 mL from the IVIG-nonresponsive group within 3–5 days after the second IVIG treatment. The blood was then centrifuged at 3000 rpm for 10 min. The serum was separated from the residue and stored in a refrigerator at -80°C for testing. Then, 4 mL of fasting venous blood was drawn from all pediatric patients in the control group and processed in the same manner as for the KD group. The serum was collected, and the levels of Meprin A, IL-1 $\beta$ , and IP-10 s were detected using Meprin A ELISA kit, IL-1 $\beta$  ELISA kit, and IP-10 kit, respectively (all supplied by BD Biosciences $\boxtimes$ ).

## Treatment protocols

Standard protocol: All pediatric patients with KD were given IVIG 2 g/kg once, combined with 30–50 mg/(kg  $\times$  day) aspirin. The dosage of aspirin was reduced to 3–5 mg/kg 3 days after the abatement of fever.

Second treatment protocol: IVIG-nonresponsive group: Protocol 1 group was given IVIG 1 g/kg once again; Protocol 2 group was given IVIG 2 g/kg once again; Protocol 3 group was given IVIG 1 g/kg + Solu-Medrol injection 2 mg/kg (intravenous  $\times$ 3 days, changed to oral administration of prednisone tablets, and dosage gradually reduced until withdrawn, with a cycle of 1–2 weeks).

Third treatment protocol: If Protocol 1 group and Protocol 2 group were nonresponsive after the second treatment: Solu-Medrol injection 2 mg/kg was given (intravenous  $\times$ 3 days, changed to oral administration

of prednisone tablets, and dosage gradually reduced until withdrawn, with a cycle of 1–2 weeks; if Protocol 3 was nonresponsive, IVIG 1g/kg was administered.

The body temperatures of all the pediatric patients with KD were under control after the third treatment.

All patients were given immunoglobulins produced by the same company (Chengdu Rongsheng Pharmaceuticals Co., Ltd., under State Food and Drug Administration (SFDA) Approval No.: S19993042, 50 mL: 2.5 g/vial), aspirin enteric-coated tablets (Cisen Pharmaceutical Co., Ltd., under SFDA Approval No.: H37023270, 25 mg/tablet), Solu-Medrol injection (Pfizer Manufacturing Belgium NV, under imported drug reg. standard: JX20160069, 40 mg/vial), and prednisone acetate tablets (Zhejiang Xianju Pharmaceutical Co., Ltd., under SFDA Approval No.: H33021207, 5 mg/tablet).

## Statistical analysis

SPSS19.0 was used for statistical analysis. The normal distribution data were expressed as mean  $\pm$  standard deviation and checked using the independent-samples *t* test. The data with non-normal distribution were expressed as median (interquartile range) and compared using the Wilcoxon rank-sum test. The counting data were expressed as a percentage (%) and processed using the chi-square test. *P* value less than 0.05 was considered to be statistically significant.

## Results

### Comparison of pretreatment clinical and lab indexes between the IVIG-responsive group, IVIG-nonresponsive group, and control group

Comparison between the three groups: The IVIG-responsive group comprised 574 out of 633 patients with KD (86.4%). The IVIG-nonresponsive group comprised 86 patients (13.6%). The control group comprised 200 participants. No statistically significant difference in age and sex was found between the three groups ( $P>0.05$ ). The WBC count, ESR, platelet (PLT) count, and serum levels of CRP, Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10 were higher in the IVIG-responsive and nonresponsive groups than in the control group, and the difference was statistically significant ( $P<0.001$ ). More details are shown in Table 1.

Comparison between the IVIG-nonresponsive and IVIG-responsive groups: The CAL incidence rate was higher in the IVIG-nonresponsive group (58.1%) than in the IVIG-responsive group (12.2%;  $P<0.05$ ). The IVIG-nonresponsive group was found with longer time of first application of IVIG ( $P<0.001$ ) and higher WBC count, ESR, and levels of CRP, Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10 in the serum, with statistically significant differences ( $P<0.001$ ). More details are shown in Table 1.

# Comparison of clinical and lab indexes before and after treatment between three protocols

Comparison of Protocol 3 group with Protocol 1 and 2 groups: The differences in time of first application of IVIG and lab indexes in every acute phase were not statistically significantly different between the three groups ( $P>0.05$ ). In Protocol 3 group, the average time of fever abatement after the second treatment was 1 day, 96.7% of patients had fever abatement (responsive rate), and the CAL incidence rate after treatment was 10%; these differences were statistically significant compared with Protocol 1 and 2 groups ( $P<0.05$ ). The differences in WBC count, ESR, and serum levels of CRP, Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10 were statistically significant between Protocol 3 and 1 groups ( $P<0.05$ ). The differences in WBC count, ESR, and levels of CRP and Meprin A were statistically significant between Protocol 3 and 2 groups. More details are given in Table 2.

Comparison between Protocol 1 and 2 groups: The responsive rate (77.8%) was significantly higher in Protocol 2 group than in Protocol 1 group (50.0%). The CAL incidence rate (22.2%) after treatment was significantly lower in Protocol 2 group than that in Protocol 1 group (55.0%). The extent of decrease in serum levels of Meprin A, IL-1 $\beta$ , and IL-6 after treatment in Protocol 2 group was more obvious, and the differences were statistically significant ( $P<0.05$ ). More details are shown in Table 2.

## Comparison of clinical and lab indexes after the second treatment between responsive (Group A) and nonresponsive groups (Group B)

The 86 IVIG-nonresponsive patients with KD included 67 (77.9%) in the second treatment responsive group (Group A) and 19 (22.1%) in the second treatment nonresponsive group (Group B). In Group B, 18 (94.7%) were given IVIG-alone treatment, and 1 (5.3%) was given IVIG combined with glucocorticoids. The difference in age and sex was not statistically significant between the two groups ( $P>0.05$ ). In Group B after the second treatment, the CAL incidence rate was up to 78.9%, which was significantly higher than that in Group A ( $P<0.001$ ). The differences in serum levels of Meprin A, IL-1 $\beta$ , and IL-17A were statistically significant between the two groups ( $P<0.05$ ), but the differences in WBC count, Hb, PLT count, ESR, and levels of CRP, IL-6, and IP-10 were not statistically different ( $P>0.05$ ). All lab indexes were tested 3–5 days after the second treatment. More details are shown in Table 3.

## Discussion

KD is acute, systemic, nonspecific, self-limiting vasculitis. Its adverse effects depend mainly on the incidence of CALs, including coronary artery dilation, coronary aneurysm, thrombosis, myocardial ischemia, and even sudden death [7,8]. Intravenous immunoglobulin (IVIG) is one of the first-line drugs used to treat KD. However, the nonresponsive rate for KD treatment with IVIG continued to increase every

year, and the exposure rate to CALs was high [9,10]. In this study, the nonresponsive rate for the first IVIG treatment was 13.6% and the CAL rate was up to 58.1%, which were significantly higher than those in the first IVIG-responsive group. In the second treatment, the nonresponsive rate for the treatment with IVIG 1 g/kg alone was up to 50.0%, and the CAL rate during the follow-up visit after the second treatment was 55.0%. The nonresponsive rate for the treatment with IVIG 2 g/kg alone was still up to 22.2%, and the CAL rate during the follow-up visit after the second treatment remained 22.2%. In summary, the response of IVIG 2 g/kg to refractory KD was confirmed compared with IVIG 1 g/kg, but with a certain limitation. However, the disease mechanism of refractory KD remains unclear to date. Compared with the IVIG-responsive and control groups, the serum levels of Meprin A, IL-1 $\beta$ , IL-6, IL-17A, and IP-10 significantly increased in the IVIG-nonresponsive group ( $P<0.05$ ). After the second treatment, the serum levels of Meprin A, IL-1 $\beta$ , and IL-17A were still significantly higher in the second nonresponsive group than in the second responsive group ( $P<0.05$ ). These findings suggested that the serum levels of Meprin A, IL-1 $\beta$ , IL-17A, and other factors might influence the response to IVIG, and hence serve as a predictive index for refractory KD.

IL-1 $\beta$  plays a crucial role in the disease mechanism of KD by inhibiting the IL-1 $\beta$  pathway and blocking IL-1 $\beta$  signaling transduction, thus inhibiting the onset and progress of KD. IL-1 $\beta$  is also closely associated with the resistance to IVIG [11,12]. Recent studies also suggested that IFN- $\gamma$  and IL-6 were independent risk factors for pediatric refractory KD [13]. Meprins are zinciferous metallopeptidases with  $\alpha$  and  $\beta$  subunits [14]. Meprin A is a homodimer of  $\alpha$  subunit ( $\alpha$ - $\alpha$ ) or a heterodimer of  $\alpha$  and  $\beta$  subunits ( $\alpha$ - $\beta$ ), which can transform the precursor form of IL-1 $\beta$  (pro-IL-1 $\beta$ ) into the biologically active IL-1 $\beta$ . It not only hydrolyzes and inactivates IL-6 but also produces sIL-6R through the lysis of IL-6R on the cell membrane to mediate IL-6 countersignaling [15,16]. In addition, an experiment on mice showed that the effect of overexpressed Meprin- $\alpha$  on the formation of atherosclerosis might be closely associated with the inflammatory response mediated by active type 1 helper T cells, which facilitated the secretion of IFN- $\gamma$  [17]. Consequently, it was presumed that Meprin A might be involved in the onset and progress of refractory KD by regulating the imbalance of inflammatory mediators (e.g., IL-6 pathway) and the further release of IL-1 $\beta$  and IFN- $\gamma$ . The present study found that the serum level of IFN- $\gamma$  of patients with KD in the first IVIG-nonresponsive group induced a significant increase in the expression of IP-10. IP-10 was secreted from a variety of IFN- $\gamma$ -induced tissue cells and immune cells [18]. It exerted a recruiting and chemotactic effect on T cells in autoimmune diseases and aggregated the corresponding T cells to target organs, causing tissue and organ damage [19]. IP-10/IL-17 secreted from the plasma of pediatric patients with KD acted on the calcification of human coronary artery smooth muscle cells (HCASMCs); IL-17 might also interact with IFN- $\gamma$  to enhance the production of IP-10 [8]. Thus, it was inferred that IP-10 and IL-17A cooperatively influenced the CALs in KD. IL-17A is a member of the IL-17 family. The pro-inflammatory cytokines with one or more biological functions are produced from immune cells, such as Th17 cells, natural killer cells, and mast cells. They upregulate the expression of pro-inflammatory genes and induce the production of IL-6, IL-8, and INF- $\alpha$  in some cells (e.g., endothelial cells and macrophages) [20]. Japanese researchers also found that IL-1 $\beta$  and IL-17A are involved in IVIG resistance through activation of C/EBP $\beta$  and  $\delta$  in a coronary artery model of Kawasaki disease [1].

Glucocorticoids (GCs), as an alternative to inflammation suppression, have gained increasing attention in the treatment of refractory KD. However, their mechanism of action remains unclear. GCs can upregulate or downregulate up to 20% of gene expression [21]. They bind to cytoplasmic receptors to inhibit the transcription of inflammatory proteins but promote the transcription of anti-inflammatory proteins, thus fulfilling the anti-inflammatory role at the genome level. These proteins include cytokines such as TNF- $\alpha$ , IL-6, IFN- $\gamma$ , chemokines, and cell adhesion molecules [22]. They can also reduce the secretion of inflammatory cytokines by influencing the activity of NF- $\kappa$ B [5]. In this study, the extent of decrease in the serum levels of Meprin A, IL-1 $\beta$ , and IL-6 after the second treatment was higher in the IVIG 2 g /kg group compared with the IVIG 1 g/kg group. In the IVIG 1 g/kg + GC group, the serum levels of Meprin A, IL-1 $\beta$ , and IL-6 decreased significantly, and the extent of decrease in the IL-17A and IP-10 levels was also extremely obvious ( $P < 0.05$ ). The extent of decrease in serum levels of Meprin A after the second treatment was even higher in the IVIG 1 g/kg +GC group compared with the IVIG 2 g/kg group ( $P < 0.05$ ). Moreover, 94.7% of the patients in the second treatment nonresponsive group were given IVIG alone; serum levels of Meprin A, IL-1 $\beta$ , and IL-17A were significantly higher than that in the second treatment responsive group. The responsive rate in the IVIG-1 g/kg + GC group was up to 96.7%, and the CAL incidence during the follow-up visit after the second treatment was 10%, which was 2.2 times lower than that in the IVIG-2 g/kg group and 5.5 times lower than that in the IVIG-1 g/kg group. This suggested that GCs showed a better anti-inflammatory effect and a long-term prognosis. This might be associated with a more rapid function of GCs. Previous studies showed that IVIG combined with a GC protocol rapidly decreased the levels of inflammatory cytokines such as IL-2, IL-6, and IL-8 in the peripheral circulation within 24 h [23], when used to treat KD cases not responsive to IVIG. Reduced duration of systemic inflammation would significantly reduce the time of fever abatement and decrease the exposure rate of CAL.

## Conclusion

A systematic analysis found that IL-1 $\beta$ , Meprin A, IL-17A, and other cytokines might be the factors influencing refractory KD and CAL, which are potentially good predictors. IVIG combined with GCs responsively controlled the levels of IL-1 $\beta$ , Meprin A, IL-17, and other inflammatory factors in patients with refractory KD, reduced the incidence rate of CAL, and improved the responsive rate. This suggested that IVIG combined with GCs would have more advantages and prospects in KD treatment compared with IVIG alone. IVIG combined with a GC protocol should be given in the event of a significant increase in the levels of certain inflammatory factors such as IL-1 $\beta$ , Meprin A, and IL-17. However, this study still had limitations. The GC treatment group should be added in subsequent studies on refractory KD for the comparison of response and safety.

## List Of Abbreviations

KD    Kawasaki disease

IVIG    Intravenous immunoglobulin

WBC White blood cell

CRP C-reactive protein

ESR Erythrocyte sedimentation rate

CALs Coronary artery lesions

GC Glucocorticoid

UTI Ulinastatin

MTX Methotrexate

IFX Infliximab

IL-1 $\beta$  Interleukin-1 $\beta$

IP-10 Interferon-induced protein-10

IL-6 Interleukin-6

SFDA Tate Food and Drug Administration

PLT Platelet

Hb Hemoglobin

HCASMCs Human coronary artery smooth muscle cells

## Declarations

### **Ethics approval and consent to participate**

This study was approved by the Medical Ethics Committee of Ningbo Women and Children's Hospital (2018- LSZ-22).

### **Consent for publication**

All patients and their parents provided written informed consent for their data to be used in analyses and reported.

### **No Competing interests**

The authors declare that they have no competing interests.

### **Funding**

Medical youth talent program of Zhejiang Province (2019RC267) Ningbo leading and top talent cultivation project (NBLJ201801035); Ningbo science and technology bureau natural fund project(2016A610172).

### **Authors' contributions**

LW and FFH was responsible of the collection of clinical information on KD patients, statistical analyses, figures, data interpretation and manuscript preparation. LW and ZJC were responsible for critical review of the statistical analyses and the manuscript. LW and SLZ contributed in the writing of the manuscript and its scientific content. YZD is the project leader of the study; she is involved in the conceptualization of the project, the study design and preparation of the manuscript. All authors read and approved the final manuscript.

### **Acknowledgements**

We are very grateful to the Lab of Ningbo Women and Children's Hospital. We also want to thank all participating patients and their families for their support.

### **Authors' information (optional)**

Ling Wu Email:wuling\_ningbo@163.com

Zengjie Chen Email:294186909@qq.com

Shiling Zhong Email:184799321@qq.com

Fangfang Hu Email:2759083845@qq.com

Yazhen Di Email:dyz028sci@163.com

## **References**

1. Inoue T, Miyashita M, Murakami S, Igarashi A, Motomura K, Abe J, et al. IL-1beta and IL-17A are involved in IVIG resistance through activation of C/EBPbeta and delta in a coronary artery model of Kawasaki disease. *Allergy*. 2020. doi:10.1111/all.14281.
2. Zhao J, Huang M, Xie L, et al. Clinical analysis of initial treatment of kawasaki disease with glucocorticoid. *International Journal of Pediatrics*. 2017;44:642–5,649.
3. Ishii M, Ebato T, Kato H. and Future of Treatment for Acute Stage Kawasaki Disease. *Korean Circ J*. 2020;50:112–9.
4. Chang LS, Kuo HC. The role of corticosteroids in the treatment of Kawasaki disease. *Expert Rev Anti Infect Ther*. 2020;18:155–64.
5. Zhen L, Duan C-C, Zhao D-Y, et al. Study on the therapeutic effect of hormone in ag-globulin nonreactive kawasaki disease. *Journal of cardiopulmonary vascular disease*. 2019;38:833–7.

6. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, 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:e927–99.
7. Zheng X, Zhang Y, Liu L, Yue P, Wang C, Zhou K, et al. N-terminal pro-brain natriuretic peptide as a biomarker for predicting coronary artery lesion of Kawasaki disease. *Sci Rep*. 2020;10:5130.
8. Chang SF, Liu SF, Chen CN, Kuo HC. Serum IP-10 and IL-17 from Kawasaki disease patients induce calcification-related genes and proteins in human coronary artery smooth muscle cells in vitro. *Cell Biosci*. 2020;10:36.
9. Soni PR, Noval Rivas M, Arditi M. A Comprehensive Update on Kawasaki Disease Vasculitis and Myocarditis. *Curr Rheumatol Rep*. 2020;22:6.
10. Chen Y, Hua Y, Zhang C, Chen S, Zhang Q, Liao Y, et al. Neutrophil-to-Lymphocyte Ratio Predicts Intravenous Immunoglobulin-Resistance in Infants Under 12-Months Old with Kawasaki Disease. *Front Pediatr*. 2019;7:81.
11. Takeshita S, Kanai T, Kawamura Y, Yoshida Y, Nonoyama S. A comparison of the predictive validity of the combination of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio and other risk scoring systems for intravenous immunoglobulin (ivig)-resistance in Kawasaki disease. *PLoS One*. 2017;12:e0176957.
12. Hashimoto Y, Fukazawa R, Nagi-Miura N, Ohno N, Suzuki N, Katsube Y, et al. Interleukin-1beta Inhibition Attenuates Vasculitis in a Mouse Model of Kawasaki Disease. *J Nippon Med Sch*. 2019;86:108–16.
13. Wang Y, Yun Qian S, Yuan Y, Wang Q, Gao L, Chen X, et al. Do Cytokines Correlate with Refractory Kawasaki Disease in Children? *Clin Chim Acta*. 2020;506:222–7.
14. Gooding J, Cao L, Whitaker C, Mwiza JM, Fernander M, Ahmed F, et al. Meprin  $\beta$  metalloproteases associated with differential metabolite profiles in the plasma and urine of mice with type 1 diabetes and diabetic nephropathy. *BMC Nephrol*. 2019;20:141.
15. Peters F, Becker-Pauly C. Role of meprin metalloproteases in metastasis and tumor microenvironment. *Cancer Metastasis Rev*. 2019;38:347–56.
16. Herzog C, Haun RS, Kaushal GP. Role of meprin metalloproteinases in cytokine processing and inflammation. *Cytokine*. 2019;114:18–25.
17. Lu T, Tang G, Si L-Y, Gao P. Study on the role of Meprin A in regulating T cell activation-mediated atherosclerosis. *Chinese journal of cardiovascular cerebrovascular diseases in the elderly*. 2017;19:74–7.
18. Hachiya A, Kobayashi N, Matsuzaki S, Takeuchi Y, Akazawa Y, Shigemura T, et al. Analysis of biomarker serum levels in IVIG and infliximab refractory Kawasaki disease patients. *Clin Rheumatol*. 2018;37:1937–43.
19. Lin Q, Song Y-F. The role of ip-10 in the pathogenesis of autoimmune diseases and its application prospect. *Chinese journal of laboratory medicine*. 2015;38:805–8.

20. Jaszczura M, Mizgała-Izworska E, Świętochowska E, Machura E. Serum levels of selected cytokines [interleukin (IL)-17A, IL-18, IL-23] and chemokines (RANTES, IP10) in the acute phase of immunoglobulin A vasculitis in children. *Rheumatol Int.* 2019;39:1945–53.
21. Cain DW, Cidlowski JA. Specificity and sensitivity of glucocorticoid signaling in health and disease. *Best Pract Res Clin Endocrinol Metab.* 2015;29:545–56.
22. Miura M. Role of glucocorticoids in Kawasaki disease. *Int J Rheum Dis.* 2018;21:70–5.
23. Kobayashi T, Kobayashi T, Morikawa A, Ikeda K, Seki M, Shimoyama S, et al. Efficacy of intravenous immunoglobulin combined with prednisolone following resistance to initial intravenous immunoglobulin treatment of acute Kawasaki disease. *J Pediatr.* 2013;163:521–6.

## Tables

**Table 1.** Comparison of pretreatment clinical and lab indexes between the IVIG-responsive group, IVIG-nonresponsive group, and control group

	IVIG-responsive group	IVIG-nonresponsive group	Control group	$\chi^2/Z$ value	Pvalue
n/%	547.0(86.4)	86(13.6)	200		
Men, n (%)	298(54.5)	48(54.7)	108(54.0)	0.08	0.961
Age (year)	2.0(1.3)	2.0(1.3)	1.5(1.3)	4.377	0.112
Time of first application of IVIG(day)	5(5.7)	6(5.8)		-4.631	0.000
CAL(%)	67(12.2)	50(58.1)		103.847	0.000
WBC ( $10^9/L$ )	14.2(12.6-17.5)	18.0(14.1-21.6)*	6.8(4.7-10.0)	389.367	0.000
Hb (g/L)	10.8(10.2-11.4)	10.6(10.2-11.2)	10.8(10.1-11.5)	0.871	0.647
PLT ( $10^9/L$ )	350(303-426)	383(329-433)	237(184-298)	188.15	0.000
CRP (mg/L)	51.2(32.1-84.1)	70.1(60.5-121.4)*	11.0(6.5-24.0)	377.598	0.000
ESR (mm/H)	58(45-74)	69(56-91)*	12(8-19)	456.263	0.000
Meprin A (ng/L)	5.2(2.5-11.1)	25.0(15.0-33.9)*	1.4(0.9-2.3)	400.862	0.000
IL-1 $\beta$ (pg/mL)	2.5(1.8-3.5)	5.1(3.2-6.1)*	1.8(1.5-2.1)	200.97	0.000
IL6 (pg/mL)	8.6(5.3-11.0)	13.5(11.0-17.7)*	4.1(2.0-7.0)	265.099	0.000
IL17A (pg/mL)	23(14-28)	29(24-35)*	8(5-11)	368.47	0.000
IP-10 (pg/mL)	271(179-339)	298(238-371)*	138(96-201)	192.499	0.000

Time of first application of IVIG: Time from fever to the first application of IVIG.

\* P < 0.05, IVIG-responsive group vs IVIG-nonresponsive group.

Table 2. Comparison of clinical and lab indexes before and after treatment between the three protocols

	Protocol 1	Protocol 2	Protocol 3	$\chi^2/Z/F$	P Value
					Value
<i>n</i>	20	36	30		
Men, <i>n</i> (%)	11(55)	20(55.6)	16(53.3)	0.034	0.983
Age [year]	2.4 ± 1.5	2.5 ± 1.5	2.4 ± 1.4	0.04	0.961
Time of first application of IVIG [day]	6.6 ± 1.8	6.4 ± 1.5	6.7 ± 1.6	0.259	0.772
Post-treatment CAL [%]	11(55.0)	8(22.2)	3(10.0)	13.131	0.001
Thermal abatementcases [%]	10(50.0)	28(77.8)	29(96.7)	15.184	0.001
Thermal abatementtime [day]	2.5(2-3)	2(1.5-2)	1(1-1)	32.449	0.000
Pretreatment WBC (10 <sup>9</sup> /L)	12.2 ± 2.4	12.0 ± 3.6	11.9 ± 4.3	0.041	0.96
Post-treatment WBC (10 <sup>9</sup> /L)	9.7 ± 2.0 <sup>#</sup>	9.8 ± 2.6 <sup>Δ</sup>	12.1 ± 2.4	9.437	0.000
Pretreatment Hb (g/L)	10.0 ± 0.9	9.9 ± 1.0	10.0 ± 0.9	0.235	0.791
Post-treatment Hb (g/L)	9.5 ± 0.7	9.4 ± 0.7	9.7 ± 1.7	0.606	0.548
Pretreatment PLT (10 <sup>9</sup> /L)	499.7 ± 53.2	491.2 ± 70.6	498.4 ± 74.0	0.135	0.874
Post-treatment PLT (10 <sup>9</sup> /L)	595.1 ± 74.7	612.0±81.0	639.2±133.2	1.236	0.296
Pretreatment CRP (mg/L)	38.4±15.4	43.0 ± 20.0	40.3 ± 20.2	0.409	0.666
Post-treatment CRP (mg/L)	21.0 ± 11.6 <sup>#</sup>	23.1 ± 9.8 <sup>Δ</sup>	14.0 ± 6.0	8.306	0.001
Pretreatment ESR (mm/H)	82.1 ± 10.9	83.7 ± 20.7	77.8 ± 22.4	0.766	0.468
Post-treatment ESR (mm/H)	98.1 ± 10.0 <sup>#</sup>	104.9 ± 17.1 <sup>Δ</sup>	84.0 ± 12.4	18.225	0.000
Pretreatment Meprin A (ng/L)	13.9 ± 6.6	13.7 ± 6.2	12.0 ± 4.9	0.859	0.427
Post-treatmentMeprin A (ng/L)	11.7 ± 4.5 <sup>#</sup>	9.3 ± 2.8 <sup>Δ</sup>	6.1 ± 2.0	21.18	0.000

Pretreatment IL-1 $\beta$ (pg/mL)	3.0 $\pm$ 0.8	2.8 $\pm$ 0.8	2.9 $\pm$ 0.9	0.442	0.644
Post-treatment IL-1 $\beta$ (pg/mL)	2.6 $\pm$ 0.6 <sup><math>\phi</math>#</sup>	1.8 $\pm$ 0.5	1.6 $\pm$ 0.5	20.758	0.000
Pretreatment IL6 (pg/mL)	9.0 $\pm$ 2.6	8.8 $\pm$ 2.8	8.9 $\pm$ 3.1	0.024	0.976
Post-treatment IL6 (pg/mL)	7.4 $\pm$ 2.5 <sup><math>\phi</math>#</sup>	5.2 $\pm$ 1.6	5.3 $\pm$ 2.2	8.623	0.000
Pretreatment IL-17A (pg/mL)	26.3 $\pm$ 5.8	25.1 $\pm$ 7.3	23.0 $\pm$ 9.4	1.112	0.334
Post-treatment IL-17A (pg/mL)	17.6 $\pm$ 6.0 <sup>#</sup>	14.6 $\pm$ 4.8	12.2 $\pm$ 2.8	8.494	0.000
Pretreatment IP-10 (pg/mL)	236.3 $\pm$ 55.5	233.2 $\pm$ 85.6	210.5 $\pm$ 83.2	0.897	0.412
Post-treatment IP-10 (pg/mL)	185.7 $\pm$ 45.0 <sup>#</sup>	168.1 $\pm$ 66.8	138.9 $\pm$ 37.1	5.049	0.009

Thermal abatement cases: The number of cases in which the body temperature was stable and no longer repeated after the second treatment.

The time of fever abatement: The time from the day of second treatment to resumption of normal body temperature without a recurrent fever.

Post-treatment CAL: The CAL appearing during the follow-up visit after the second treatment, excluding that found before the second treatment.

Pretreatment: Prior to the second IVIG treatment, within 3-5 days after the first IVIG treatment.

Post-treatment: Within 3-5 days after the second IVIG treatment.

<sup>$\phi$</sup>  $P < 0.05$ , Protocol 1 vs Protocol 2; <sup>#</sup>  $P < 0.05$ , Protocol 1 vs Protocol 3;  <sup>$\Delta$</sup>  $P < 0.05$ , Protocol 2 vs Protocol 3.

**Table 3.** Comparison of clinical and lab indexes after the second treatment between the responsive (Group A) and nonresponsive groups (Group B)

	Group A	Group B	$\chi^2/Z/F$	P Value
				Value
N (%)	67 (77.9)	19 (22.1)		
Men, n (%)	36 (53.7)	11 (57.9)	0.104	0.748
Age [year]	2 (1-3)	2 (2-3)	-0.249	0.804
Post-treatment CAL [%]	7 (10.4)	15 (78.9)	32.974	0.000
WBC ( $10^9/L$ )	11.7 ± 3.6	12.9 ± 3.5	-1.307	0.195
Hb (g/L)	9.9 ± 0.9	10.2 ± 1.1	-1.355	0.188
PLT ( $10^9/L$ )	495.1 ± 71.6	497.6 ± 52.8	-0.143	0.887
CRP (mg/L)	40.3 ± 19.2	43.6 ± 18.3	-0.685	0.495
ESR (mm/H)	80.3 ± 20.3	84.5 ± 16.6	-0.823	0.413
Meprin A (ng/L)	11 (8-12)	19 (15-23)	-5.293	0.000
IL-1 $\beta$ (pg/mL)	2.8 ± 0.8	3.3 ± 0.8	-2.802	0.006
IL6 (pg/mL)	8 (7-9)	8 (6-12)	-0.38	0.704
IL-17A (pg/mL)	22.4 ± 7.0	32.6 ± 5.3	-5.917	0.000
IP-10 (pg/mL)	225.2 ± 82.3	228.7 ± 66.6	-0.167	0.867