# PATHOGENESIS, CLINICAL FINDINGS AND TREATMENT ADVANCES IN KAWASAKI DISEASE

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# PATHOGENESIS, CLINICAL FINDINGS AND TREATMENT ADVANCES IN KAWASAKI DISEASE

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# Table of Contents

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EDITORIAL
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# Editorial: Pathogenesis, Clinical Findings, and Treatment Advances in Kawasaki Disease

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**Keywords: Kawasaki, coronary, coronary arterial lesions, aneurysm, vasculitis**

## Editorial on the Research Topic

## Pathogenesis, Clinical Findings, and Treatment Advances in Kawasaki Disease

The recent Research Topic on Kawasaki disease (KD) covers some interesting issues; some have been dealt with during the first European meeting on KD, which was held in Paris in January 2021 and which was organized by two of the authors (RC and IKP). The topic includes 11 articles, and covers epidemiology ($n$ = one article), etiopathogenesis ($n = 2$), clinical aspects ($n = 3$), imaging ($n = 1$), treatment ($n = 2$), and the "hot" subject of COVID19-related multisystem inflammatory syndrome in children (MIS-C) ($n = 2$).

The epidemiology of KD varies throughout the world. The disease is well-known to be more frequent in Asia, with figures more than 20-fold higher in Japanese when compared to Caucasians. Piram in her article showed that the annual incidence of KD in northern and western European countries is about 10–15 per 100,000 children under 5 years of age and seems to be relatively stable over time. From the limited data available, the incidence seems to be similar in Eastern and Western Europe. Demographic characteristics of KD in Europe are in line with those in other countries.

Although the exact pathogenesis of KD is still unclear, possible triggers may be viral infections, which remain most often unidentified. According to a study, systemic immune responses early in life could protect against developing KD, so the incidence of previous Cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infections was investigated in children with KD compared to healthy age-matched controls (van Stijn et al.). By comparing 86 KD patients with an age-matched group with regard to CMV and EBV VCA IgG measurements (taken before or 9 months after IVIG treatment), the authors found that both CMV and EBV had an almost 2-fold lower seroprevalence in the KD population than in the control group.

The group of Arditi in Los Angeles provided an interesting paper on an animal model (Maruyama et al.). miRNAs inhibit mRNA translation or decrease the levels of their corresponding mRNA, thereby inhibiting targeted protein synthesis. miR-223 has been shown to be up-regulated in the blood and coronary tissue of KD patients. This study shows that miR-223 expression is up-regulated in inflamed aneurysms and dilatations of LCWE-injected mice, and that mice lacking miR-223 have more vessel inflammation and IL-1 beta secretion. Thus, the authors have hypothesized that intense IL-1 beta production during LCWE-induced KD vasculitis induces miR-223 expression as a negative regulatory feedback loop.

Concerning clinical aspects, the criteria that we still rely on are those endorsed by AHA and AAP (1). However, in many cases the diagnosis might be difficult, since KD may mimic viral infections and other inflammatory disorders such as systemic onset JIA (sJIA). Go et al. suggested that sJIA and KD might belong to the same spectrum of IL-1 mediated cytokine storm syndromes. They evaluated in a large retrospective study of patients with KD ($n$ = 1,765) and sJIA ($n$ = 112) the

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frequency of patients combining the two diagnoses (KD/sJIA). Seven percent (75% males) fulfilled both criteria; they had a median age of 4.7 years, hence higher than that of KD patients. KD/sJIA had less conjunctival injection and half had incomplete KD features. They also received more than one IVIg infusion and 62.5% had coronary dilatation. In these patients, arthritis appeared between 1 and 4 months after the onset of symptoms.

Of note, arthritis is a complication of KD that occurs in up to one third of untreated patients. In order to stratify patients for risk factors and therefore for treatment selection during the course of KD, a nationwide survey in Japan was conducted on patients who developed arthritis requiring a treatment (Kanemasa et al.). The serum levels of ferritin and IL-18 or the combination of clinical covariates at the onset of arthritis could predict the treatment response and prognosis of arthritis.

Being a systemic vasculitis KD can affect any organ system. However, neurologic involvement is often underdiagnosed. Maggio et al., showed that abnormalities of brainstem auditory evoked potentials were associated with the risk of coronary artery lesions. The authors suggested that this might be a concomitant subclinical vasculitis of *vasa nervorum*.

However, the more dreadful complication is undoubtedly coronary involvement; van Stijn et al., in their retrospective single-center study compared the diagnostic yield of coronary computed tomographic angiography and cardiac magnetic resonance imaging (MRI) for the detection of coronary abnormalities. Coronary computed tomographic angiography could assess the coronary artery tree at great resolution, identifying coronary artery aneurysms more frequently and with greater detail when compared to cardiac MRI.

We cannot ignore in this pandemic time the subject of MIS-C (2, 3); indeed, two articles tried to analyze the differences and similarities between this entity and KD. A Spanish study has compared the KD cases diagnosed according to the AHA criteria during the COVID-19 period (March–May, 2020) that were either SARS-CoV-2 confirmed (CoV+) or negative (CoV–) by PCR and serology, to those from the same period during 2018 and 2019 (PreCoV) (Fernández-Cooke et al.). The CoV+ group included a significantly higher proportion of non-Caucasians (64%) than Caucasians (25%). Cases and intensive care admissions increased significantly in 2020 during the CoVID-19 period (CoV−20% and CoV+ 50%, $P < 0.001$, vs. 1.6% of patients during the PreCoV).

Moreover, a Japanese observational study aimed to identify similarities and differences between Kawasaki Disease Shock Syndrome (KDSS) and MIS-C (Suzuki et al.). KDSS was more likely to have a diagnosis of complete KD, a higher incidence of coronary artery abnormalities (CAAs) (50 vs. 11%) and a greater requirement for vasoactive agonists. 6.7% of patients with KDSS died and 1.7% of patients with MIS-C died.

Finally, treatment issues have also been dealt with, in particular regarding the use of intravenous immunoglobulins (IVIG). In a Japanese study, a machine learning method used commonly available clinical and laboratory variables, in order to predict resistance to IVIG therapy (Kuniyoshi et al.). Three models based only on demographics and routine laboratory variables did not provide reliable performances. Since the identification of high-risk patients is crucial for early aggressive treatment, we still need further research including the addition of biomarkers to the model.

Lastly, in a systematic review and meta-analysis investigating the effects of early IVIG therapy (administration <5 days from disease onset), 14 studies involving 70,396 patients were included (Yan et al.). Early treatment with IVIG can lead to an increased risk of IVIG unresponsiveness [OR 2.24; 95% CI (1.76, 2.84); $p < 0.001$]. However, in contrast to studies performed in Japan that found no significant difference in coronary artery lesions (CAL) development, studies conducted in China and in the United States showed a reduced risk in the occurrence of CAL with early IVIG treatment.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SYSTEMATIC REVIEW
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# Effect of Early Intravenous Immunoglobulin Therapy in Kawasaki Disease: A Systematic Review and Meta-Analysis

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**Background:** In the latest 2017 American Heart Association guidelines for Kawasaki disease (KD), there are no recommendations regarding the early administration of intravenous immunoglobulin (IVIG). Therefore, the purpose of this systematic review and meta-analysis was to investigate the effects of early IVIG therapy on KD.

**Methods:** We searched databases including the PubMed, Medline, the Cochrane Library, and the Clinicaltrials.gov website until July 2019.

**Results:** Fourteen studies involving a total of 70,396 patients were included. Early treatment with IVIG can lead to an increased risk of IVIG unresponsiveness [OR 2.24; 95% CI (1.76, 2.84); $P = 0.000$]. In contrast to the studies performed in Japan [OR 1.27; 95% CI (0.98, 1.64); $P = 0.074$] that found no significant difference in coronary artery lesions (CAL) development, studies conducted in China [OR 0.73; 95% CI (0.66, 0.80); $P = 0.000$] and the United States [OR 0.50; 95% CI (0.38, 0.66); $P = 0.000$] showed a reduced risk in the occurrence of CAL with early IVIG treatment.

**Conclusions:** At present, the evidence does not support the treatment with IVIG in the early stage of the onset of KD. But, early IVIG treatment could be a protective factor against the development of CAL, which needs to be further clarified.

**Keywords: Kawasaki disease, intravenous immunoglobulin therapy, coronary artery lesion, coronary artery aneurysm, IVIG unresponsiveness**

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## INTRODUCTION

Kawasaki disease (KD) is an acute self-limiting disease leading to vasculitis that predominantly affects infants and young children. Coronary artery lesion (CAL) is the most common complication. In severe cases, giant coronary artery aneurysms or coronary artery ectasia can develop, which is the leading cause of acquired heart disease (1, 2). Intravenous immunoglobulin (IVIG) therapy is the first-line treatment of KD with well-established therapeutic effects in preventing coronary artery abnormalities (3). Coronary artery aneurysms (CAAs) develop in ∼25% of untreated patients (4); however, in patients receiving a timely high dose of IVIG, it is reported that only about 5% of patients (5–7).

The mechanism as to why IVIG is effective in the treatment of KD is unknown. IVIG is considered to reduce the prevalence of CAL by regulating the immune system, including the modulation of cytokine production, neutralization of bacterial superantigens or other etiologic agents, and suppression of endothelial cell activation. The 2004 American Heart Association (AHA) guidelines of KD stated that IVIG was recommended to be used within 10 days after the onset of the disease and, if possible, within seven days. And, according to a study by Muta and Fong, early administration of IVIG (within 5 days) seemed to show no significant improvement in the cardiovascular outcomes, but there was an increased need for IVIG retreatment (4, 8, 9). In the latest AHA guidelines for KD in 2017, it is suggested that for experienced clinical experts, the diagnosis of KD can be made as early as 3 days after the onset of disease if typical symptoms are present; however, there is no guideline on the timing of IVIG administration and if it can be given earlier (10).

Therefore, in our research, studies meeting the inclusion criteria were included to compare the outcomes of KD children treated with IVIG at early and routine time, including coronary artery outcome and non-response to IVIG, so as to understand the effects of early IVIG therapy in KD and to provide evidence on the timing of IVIG administration.

# METHODS

This systematic review and meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (11) (**Supplementary Checklist 1**).

## Search Strategy

We searched databases including the PubMed, Medline, the Cochrane Library, and the Clinicaltrials. gov website until July 2019 without language restrictions. The keywords and subject terms were ("immunoglobulin" OR "IVIG" OR "immune globulin") and ("Kawasaki disease" OR "Mucocutaneous Lymph Node Syndrome" OR "Kawasaki Syndrome").

## Study Selection Criteria

The population, intervention, comparison, and outcome approach was used for the study inclusion. The population of interest were children diagnosed with KD. The intervention of interest was the treatment using IVIG as the initial therapy and studies had to include a comparison between the early treatment with IVIG (IVIG administration $<$5 days of onset of disease) and $\geq$5 days of KD onset. Outcome measurements included the incidence of CAL, IVIG unresponsiveness, and CAA.

Meanwhile, the studies included fulfilled the following criteria: (1) sufficient data to extract the number of cases and controls in each group or provision of odds ratios (ORs) with 95 % confidence intervals (CIs) and (2) the types of studies should be randomized controlled or non-randomized controlled clinical trials, case-control, cross-sectional, or cohort studies.

## Data Collection and Assessment of Study Quality

For each eligible study, the following information was independently extracted by two researchers (FY and HZ): last name of first author, study design, duration of follow-up, study duration, study location, age of participants, proportion of males, sample size in each group (subgroup by time of IVIG treatment), diagnostic criteria of CAL, definition of IVIG unresponsiveness KD, outcomes, and quality criteria. The quality of the included studies was also independently assessed by two researchers (FY and HZ) using the Newcastle-Ottawa scale (12) (**Supplementary Tables 1**, **2**). When disagreements arose, group discussions would be carried out to resolve it.

## Outcome Measures

The primary outcomes were CAL and IVIG unresponsiveness. CAL included the coronary artery dilation, CAA, and coronary artery stenosis meeting the definition of the Japanese Ministry of Health criteria, coronary artery Z score system, or Chinese literature (13–15). IVIG unresponsiveness was defined as a persistent or recrudescent fever in a period after the completion of the first IVIG infusion. The secondary outcome was CAA.

## Statistical Analysis

We estimate the pooled odds ratio (OR) with a 95% confidence interval (CI) using study-specific ORs abstracted or calculated from raw data; results from a multivariate analysis or adjusted ORs were preferred to those of univariate analysis or crude results. Considering the within– and between–study variability, the random effects model was chosen. Heterogeneity among the studies was assessed using the $Q$ test. It was quantified by $I^2$ values and 25, 50, and 75% were considered as low, moderate, and high levels of heterogeneity, respectively. To test the publication bias, the Begg's and Egger's tests were used. $P < 0.1$ indicated that a publication bias existed. Sensitivity analysis was performed by evaluating the pooled estimate after omitting a study each time. Additionally, a subgroup analysis and meta-regression was conducted to detect the potential factors for heterogeneity. Statistical analysis was performed using the STATA version 15 (StataCorp, College Station, TX).

# RESULTS

## Search Results

After searching for and removing the duplicates, a total of 3,351 titles and abstracts of relevant articles were checked. After the above screening, 59 articles were read in full and 15 articles met the inclusion criteria. Among them, the participants included in the study of Muta and Abrams overlapped (8, 16). Since the subjects included in Abrams had a larger time frame, the study of Muta was excluded. The final 14 studies were enrolled in this meta-analysis. The flow chart of the included studies is shown in **Figure 1**. The characteristics of the 14 enrolled studies involving a total of 70,396 patients are summarized in **Table 1** (9, 16–28). The outcomes of the eight studies included the effects of early IVIG administration on the occurrence of CAL (17–21, 26–28),

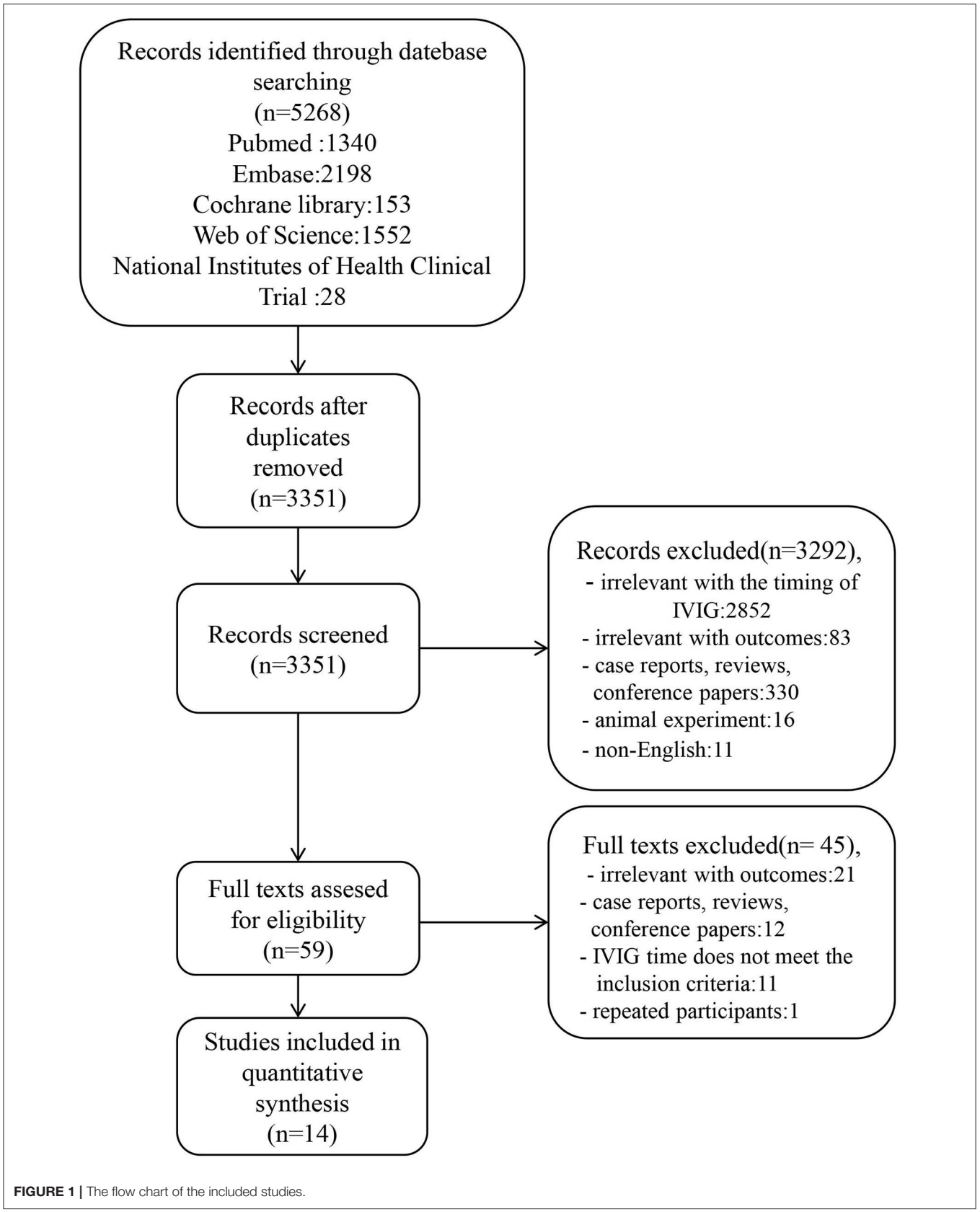


**FIGURE 1 |** The flow chart of the included studies.

**TABLE 1 |** Summary of the included studies for quantitative synthesis.

| References | Study design | Duration of following-up | Study duration | Study location | Year | Male(%) | Sample size in each group(time of IVIG), No. | Diagnostic criteria of CAL | Define of IVIG-resistant KD | Outcomes | NOS score(stars) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chen et al. (17) | Retrospectively study | NR | 2008–2012 | China | 32 days to 11.7 years | 1442 (62.6%) | <5 d 289 5–10 d 1726 >10 d 157 | Japanese criteria | NR | CAL IVIG Unresponsiveness, | 5 |
| Masanari et al. (18) | Cohort study | Over 1 month | 2011–2012 | Japan | IVIG<5 d 783 days (mean) IVIG 5–7 d 977 days (mean) IVIG>7 d 1,337 days (mean) | IVIG<5 d 41% IVIG 5–7 d 43% IVIG>7 d 40% | <5 d 6926 5–7 d 13295 >7 d 624 | Japanese criteria | NR | CAL IVIG Unresponsiveness Additional IVIG therapy | 6 |
| Shiozawa et al. (19) | Retrospectively study | 1 month | 2006–2013 | Japan | 10–36.5 months | 128 (64%) | IVIG < 5 d 100 IVIG = 5 d 100 | Japanese criteria | Need for additional treatment because of persistent fever or relapsing fever associated with other KD symptoms after resolution of fever | CAL Additional IVIG therapy IVIG-resistance | 8 |
| Abrams et al. (16) | Retrospectively study | NR | 1997–2004 | Japan | <18 years | NR | <5 d 14134 5–10 d 34176 | NR | NR | Additional IVIG, Giant aneurysms, All aneurysms, CAL, Major cardiac complications | 6 |
| Du et al. (20) | Cohort study | 6 weeks | 2000–2004 | China | 2 months–l3 year | 680 (64.6%) | <5 d 108 5–9 d 763 ≥10 d 181 | NR | Persistent fever (>38.5°C) lasted more than 48 h or recrudescent fever associated with KD symptoms after the first IVIG infusion | CAL IVIG Unresponsiveness, Aneurysm | 6 |
| Callinan et al. (21) | Retrospectively study | NR | 2000–2009 | America | <18 years | 1106 (60.6%) | <5 d 433 ≥5 d 1273 | NR | NR | CAL | 6 |
| Kobayashi (22) | Retrospectively study | 30 days | 2000-2006 | Japan | 1–119 months | 315 (58%) | <5 d NR ≥5 d NR | Japanese criteria | Persistent fever lasted more than 24 hours or recrudescent fever associated with KD symptoms after an afebrile period. | IVIG Unresponsiveness, | 6 |
| Fu et al. (23) | Retrospectively study | NR | 2002–2010 | China | 2 months−14 years | 746 (63.4%) | <5 d NR 5–10 d NR | Z scores | Persistent or recurrent fever at any time 48 h to 2 weeks after initial IVIG treatment and with at least 1 of the standard diagnostic criteria of KD | IVIG Unresponsiveness, | 5 |

*(Continued)*

**TABLE 1** | Continued

| First author, Year | Study design | Duration of following-up | Study duration | Study location | Year | Male(%) | Sample size in each group(time of IVIG), No. | Diagnostic criteria of CAL | Define of IVIG-resistant KD | Outcomes | NOS score(stars) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Egami et al. (24) | Retrospectively study | 1 month | 1998–2004 | Japan | ≤6 months 34 7–60 months 258 ≥61 months 28 | 183 (57,2%) | <5 d NR ≥5 d NR | Japanese criteria | Responder as a patient who showed resolution of fever (<37.5°C) and a fall in CRP by more than 50% within 48 h after initial IVIG treatment | IVIG Unresponsiveness | 6 |
| Fong et al. (9) | Case–control study | After the diagnosis of KD, at weeks 2, 4, and 8 and yearly | 1994–1999 | Hong Kong | <5 d 31.8 months (mean) 5–10 d 24.2 months (mean) | 50(61.7%) | <5 d 15 5–10 d 66 | NR | NR | Persistent fever, Coronary aneurysm, Additional doses of IVIG infused | 8 |
| Tremoulet et al. (25) | Retrospectively study | At the time of KD diagnosis and 2–4 weeks | 1998-2006 | Amerlca | 2.3 years (mean) | NR | <5 d NR 5–10 d NR | NR | Persistent or recrudescent fever (T ≥ 100.4°F rectally or orally) at least 48 h but not longer than 7 days after completion of the first IVIG infusion | IVIG-resistance | 6 |
| Li et al. (26) | Retrospectively study | >8 weeks | 2008–2012 | China | 3 months−16.3 years | 321(60.9%) | <5 d 131 5–10 d 293 >10 d 103 | Chinese criteria | Persistent fever that lasted more than 36 h or recrudescent fever associated with KD symptoms after the first IVIG infusion | CAL | 6 |
| Yong-Chao et al. (27) | Case–control study | NR | 2012–2014 | China | 1 months−16.2 years | 578 (64.6%) | <5 d 370 ≥5 d 525 | NR | Persistent fever that lasted more than 36 h or recrudescent fever associated with KD symptoms after the first IVIG infusion | CAL | 6 |
| Hsieh et al. (28) | Retrospectively study | At the time of KD diagnosis and again at weeks 2, 4, and 8 after treatment and annually | 1993-2003 | Taiwan | 2 months−7.8 years | 100 (61.7%) | <5 d 16 ≥5 d 146 | Japanese criteria | Fever persisted for _3 days after IVIG treatment | IVIG-nonresponsive, CAL, | 9 |

*NOS, Newcastle-Ottawa Quality Assessment Scale; NR, no record; CAL, coronary artery lesions; IVIG, intravenous immunoglobulin; Z scores, body surface area adjusted z-scores.*

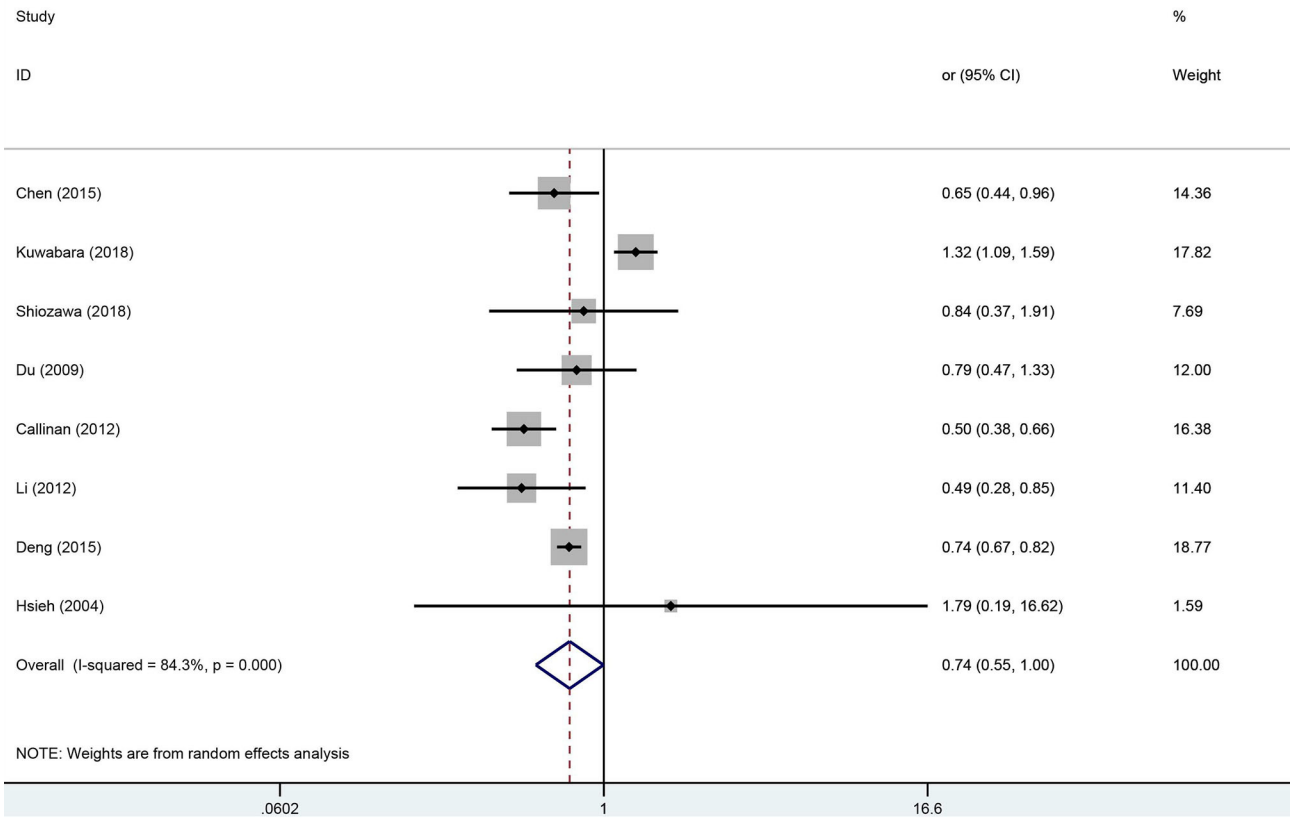


**FIGURE 2 |** Pooled odds ratio for CAL development by the timing of IVIG therapy in KD (<5 days of disease onset vs. ≥5 days).

10 studies involved IVIG unresponsiveness (9, 17–20, 22–25, 28), and CAA (9, 20) was investigated in two studies.

## Primary Outcomes

### CAL

Meta-analysis for the pooled OR in the early treatment with IVIG on CAL is shown in **Figure 2**. With upper 95% CI = 1.0, there was no evidence supporting significant differences in the incidence of CAL between the early treatment with IVIG and 5 days after onset [OR 0.74; 95% CI (0.55, 1.00); $P = 0.048$]. However, significant heterogeneity was detected between the studies ($P < 0.01$; $I^2 = 84.3\%$). Begg's test ($P = 0.266$) and Egger's test ($P = 0.263$) showed no marked asymmetry.

For sensitivity analysis, after omitting a study each time, the overall result was still stable (**Supplementary Figure 1**). To find the potential sources of heterogeneity, we used the study location, CAL diagnostic criteria, and duration of follow-up as covariates for the meta-regression analysis (**Supplementary Table 3**). The meta-regression results showed that the study location influenced the pooled estimate (China, $P = 0.003$, America, $P = 0.003$). A subgroup analysis was performed based on the study locations (**Figure 3**), we observed a significant decrease in the heterogeneity. From the overall results by each region, the Chinese studies supported that early treatment with IVIG was a protective factor against the developing CAL [OR 0.73; 95% CI (0.66, 0.80); $P < 0.001$] with a low heterogeneity ($P = 0.543$; $I^2 = 0.0\%$); however, the Japanese studies failed to show significant differences in the incidence of CAL regardless of the timing of IVIG treatment [OR 1.27; 95% CI (0.98, 1.64); $P = 0.074$]. Callinan's study was the only American study enrolled in this meta-analysis [OR 0.50; 95% CI (0.38, 0.66); $P < 0.001$].

### IVIG Unresponsiveness

Due to having a small sample size, Hsieh's study was excluded (28). Nine studies (9, 17–20, 22–25) involving IVIG unresponsiveness were included in this meta-analysis; results are shown in **Figure 4**. Early treatment with IVIG was a significant risk factor for IVIG unresponsiveness [OR 2.24; 95% CI (1.76, 2.84); $P < 0.001$]. However, a significant heterogeneity was observed ($P = 0.028$; $I^2 = 53.4\%$). Begg's test ($P = 0.917$) and Egger's test ($P = 0.877$) showed no marked asymmetry.

In the sensitivity analysis, the total findings did not change significantly (**Supplementary Figure 2**). It was noticed that Chen's study (17) reported an extremely low incidence of IVIG unresponsiveness (4.9%), which was far lower than the other studies (12.3–38.3%). After omitting this study, the heterogeneity significantly decreased [OR 2.37; 95% CI (1.95, 2.90); $P < 0.001$; $I^2 = 35.5\%$, $P = 0.145$]. A meta-regression analysis, including the definition of IVIG unresponsiveness and study location, was also

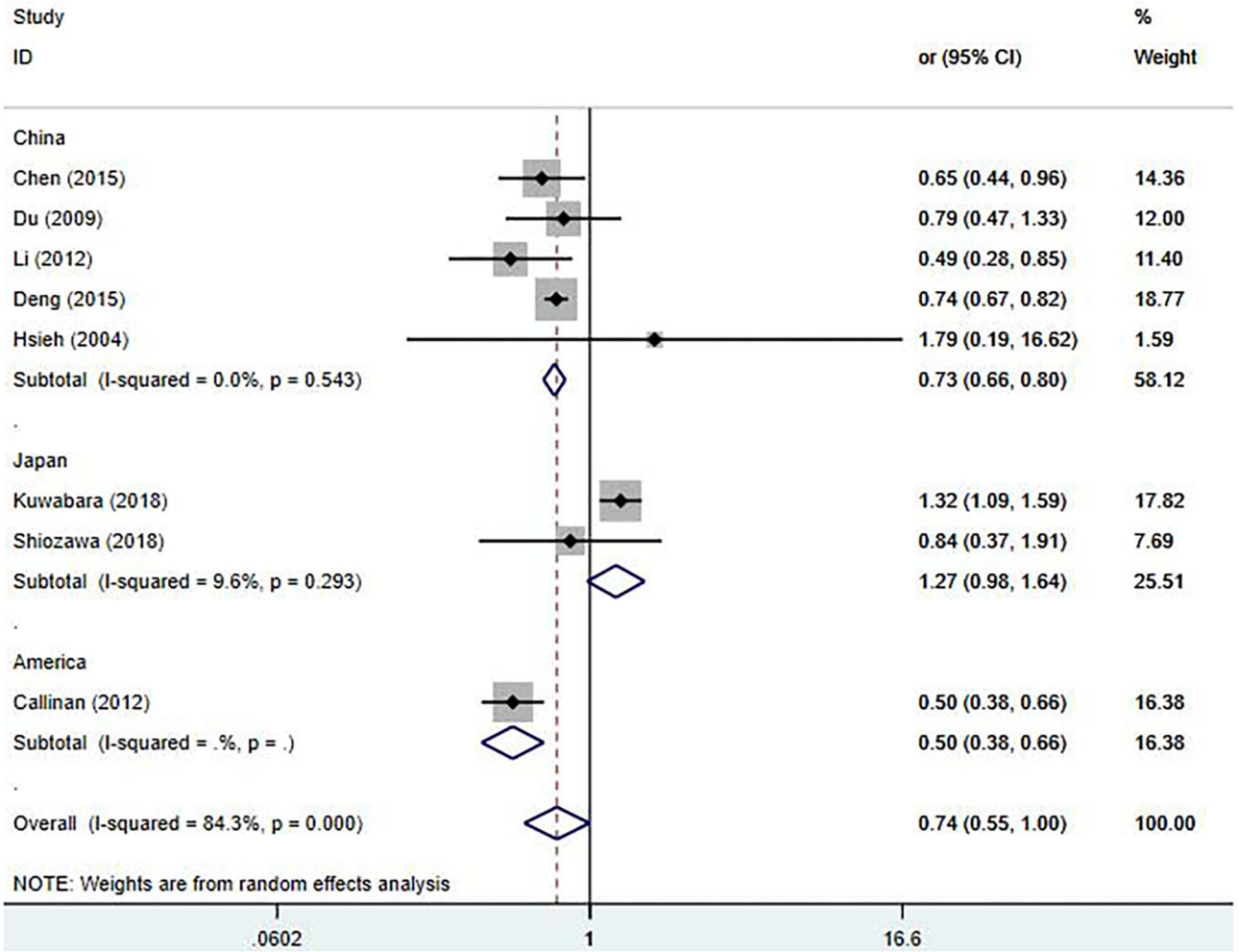


**FIGURE 3 |** Pooled odds ratio for CAL development by the timing of IVIG therapy in KD (subgroup analysis basing on the study location).

performed. However, these factors had no significant effect on the homogeneity of the included studies (**Supplementary Table 4**).

## Secondary Outcome

### CAA

Only two studies were included in the meta-analysis of the relationship between the early IVIG treatment and the development of CAA (9, 20). We found no significant differences in the incidence and a significant heterogeneity between the studies [OR 1.16; 95% CI (0.22, 6.06); $P = 0.858$; $I^2 = 56.2\%$, $P = 0.131$] (**Figure 5**).

# DISCUSSION

KD is characterized by a persistent fever in addition to five typical clinical manifestations (29) and can lead to systemic vasculitis, especially damaging the coronary arteries (30). The most effective treatment is known to be IVIG, which is recommended as a 2 g/kg single infusion 5–10 days after the onset of KD (4). Various studies have shown that the patients with a delayed IVIG treatment have a higher incidence of CAL and IVIG resistance (20, 31–33). However, current researches on the early use of IVIG is somewhat controversial.

It was reported that after the acute phase of the KD, 1,356 patients were followed-up for up to 15 years, the coronary artery events increased significantly in the patients with severe coronary dilatation. In patients with both a Z score $\geq$10 and an absolute dimension $\geq$8 mm, the incidence was as high as 48% (34). Therefore, CAL has been the focus for clinicians and researchers for a long time. Earlier literature reported that the incidence of CAL dropped from 25 to 4% after the conventional IVIG and aspirin treatment (35, 36). However, the incidence rates reported in recent years have risen significantly. In a nationwide survey on the epidemiology of KD in Japan (2011–2012), 9.3% of patients had one or more cardiac lesions (37). In 2012, Callinan reported a higher CAL incidence of 19% in California (21). Several studies

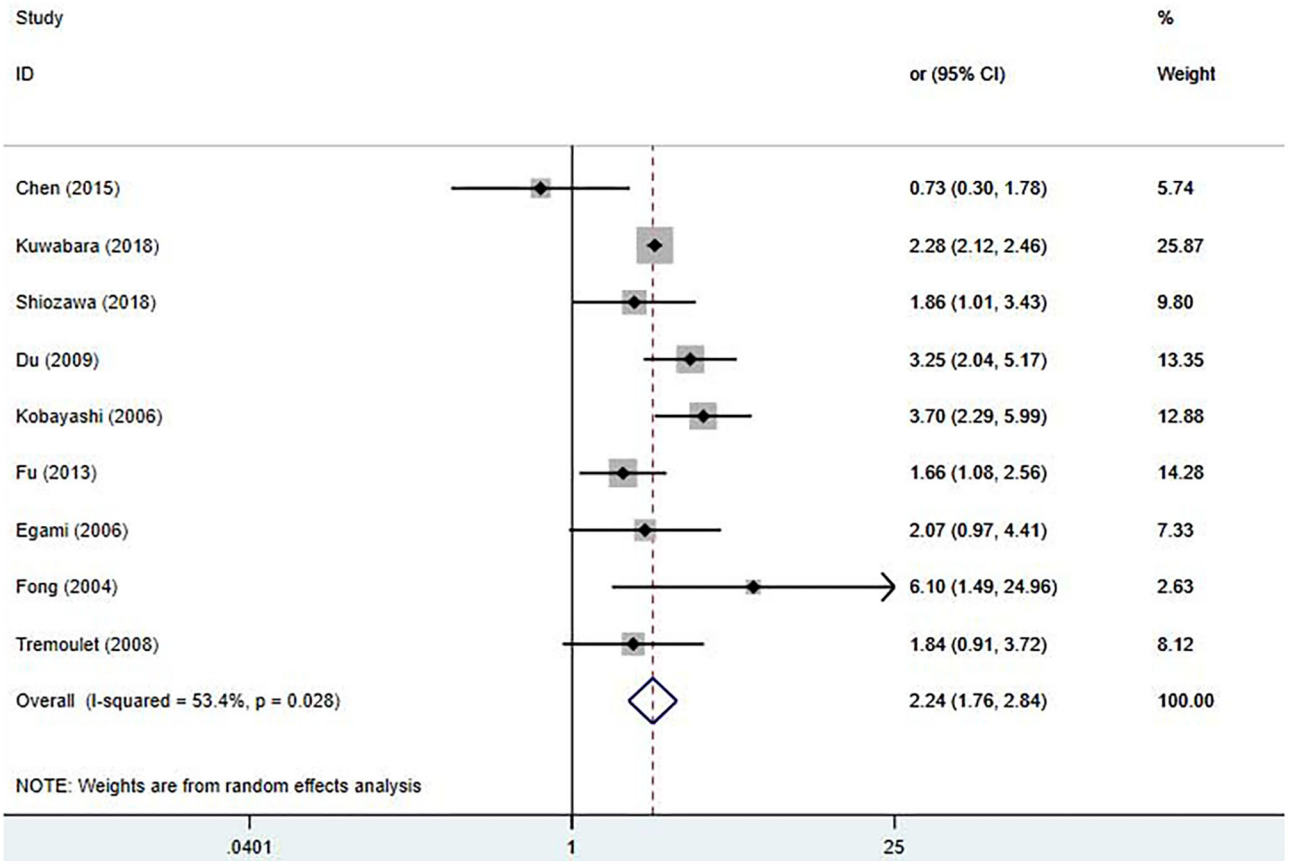


**FIGURE 4 |** Pooled odds ratio for IVIG unresponsiveness by the timing of IVIG therapy in KD (<5 days of disease onset vs. ≥5 days).

have been conducted in order to reduce the occurrence of CAL, for example involving adjunctive corticosteroid therapy, different doses of aspirin, and tumor necrosis factor inhibitors in immunoglobulin-resistant KD (3, 38, 39). Unfortunately, they all failed to find convincing positive results.

This meta-analysis was performed to investigate the effects of IVIG therapy timing on the outcomes of KD. Overall, the early use of IVIG did not significantly reduce the incidence of CAL and CAA. However, the meta-regression analysis found that the study location had a significant influence on the outcomes. In the subgroup analysis, compared to the studies done in Japan, the studies conducted in China and the United States supported early IVIG treatment as a protective factor against the development of CAL. Considering the worldwide epidemiology, the incidence of KD and CAL in East Asia is significantly higher than that in Europe and the United States, it was reported that the KD incidence per 100,000 children were reported as 264.8 cases in Japan, 134.4 cases in Korea, 82.7 in Taiwan, 51.4 in Singapore, 21.9 in Canada, 18.1 in the United States, 15.2 in Ireland, and 9 in France (40, 41). In addition, a few scoring systems used to predict the IVIG resistance are highly sensitive and specific in the Japanese population but they have not been as useful in other areas (42, 43). Thus, the characteristics of KD are thought to vary between ethnicities, which may be the reason for the difference. However, considering the study design, the results must be interpreted with caution. Part of the studies we included were a comparison of the IVIG intervention within 5 days and after 5 days, including 10 days after the KD onset. When we meta-analyzed four studies comparing IVIG treatment within 5 days and within 5–10 days, there were no significant differences in the CAL outcomes (**Supplementary Figure 3**). In addition, the different definitions of CAL and the length of follow-up after KD onset may also influence the results.

Moreover, patient-related factors are speculated to be an important risk factor for CAL development, too. Patients undergoing the early IVIG treatment may be inclined to have more typical clinical manifestations and more intense inflammatory responses than patients with the conventional or delayed IVIG treatment, leading to an early introduction of IVIG treatment that could lead to CAL development. Thus, more well-designed randomized controlled study is important for a rigorous conclusion regarding the impact of IVIG timing on the development of CAL.

The 2003–2004, 2007–2008, and 2011–2012 Nationwide Surveys in Japan reported the incidence of IVIG unresponsiveness to be 26.4, 22.1, and 17%, respectively

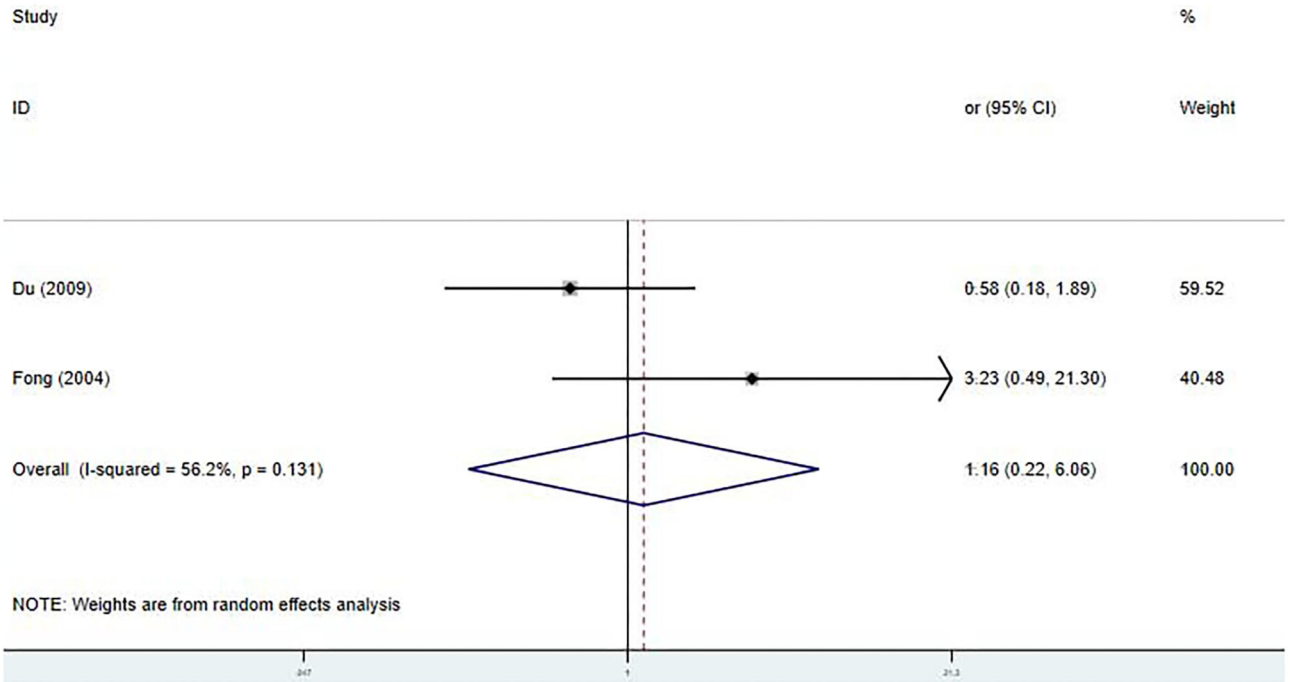


**FIGURE 5 |** Pooled odds ratio for CAA development by the timing of IVIG therapy in KD (<5 days of disease onset vs. ≥5 days).

(8, 37, 44, 45). Tremoulet reported an incidence of IVIG unresponsiveness of up to 38.3% in San Diego in 2006 (25). In a previous meta-analysis, it was found that the patients who were IVIG-unresponsive had a 3.43 times higher risk of developing CAA (46). Therefore, by the early identification of patients with IVIG unresponsiveness and active administration of additional IVIG supplemented by other treatments, the incidence of CAL may be further reduced.

Several IVIG unresponsiveness scoring systems have been published. The main factors for IVIG unresponsiveness are the days of illness at initial treatment, neutrophil percentage and count, C-reactive protein (CRP), and age (23). We also found that early infusion of IVIG is a risk factor for IVIG unresponsiveness. However, patient–related factors have been speculated to be a cause of IVIG unresponsiveness in patients who received early IVIG treatment. In the Kobayashi scoring system established in Japan, Kobayashi found that the patients in the early IVIG treatment group had higher scores regardless of the IVIG treatment time. Therefore, it is speculated that the patients receiving IVIG at an early stage may have had a more severe form of KD (22). In 2018, Shiozawa et al. conducted a study with a better design to control the impact of patients' baseline characteristics. They divided the study subjects into two groups: one group was given IVIG within 5 days after the onset of KD while the other group who were diagnosed early were not treated with IVIG until day five. They, then, adjusted for known confounders using the propensity scores. A higher rate of treatment resistance still appeared in the early treatment group (19). It was suggested that the timing of IVIG treatment influenced the reactivity to treatment regardless of the severity of KD. Besides, It was reported that in patients with KD, inflammatory mediators, including plasma white blood cell count, absolute neutrophil count, and CRP peak within 5–10 days of KD onset, thus, the inappropriate timing of IVIG treatment may lead to an increased incidence of IVIG unresponsiveness (47). However, owing to the potential confounders between groups, subsequent studies are still needed to focus on controlling for differences in patients' baseline characteristics in order to obtain reliable results.

## STUDY LIMITATIONS

Our study has several limitations. First, all included studies were non-randomized retrospective studies. Second, there were slight differences in the CAL diagnostic criteria, IVIG unresponsiveness criteria, and the time of follow-up. Third, the grouping of IVIG infusion time points in the different literatures have some differences, which limits the number of included studies. Moreover, a few studies had slightly smaller sample sizes, such as studies of Hsieh et al. and Fong et al. Thus, more randomized controlled studies with careful controlling of the confounders between groups following the standard treatment and follow-up procedures are needed to identify better time points for IVIG therapy.

## CONCLUSION

In conclusion, early treatment with IVIG can lead to an increased risk of IVIG unresponsiveness. In contrast to the

studies performed in Japan that found no significant difference in coronary outcomes, the studies conducted in China and the United States showed a reduced risk in the occurrence of CAL with an early IVIG treatment. At present, the evidence does not support the treatment with IVIG in the early stage of the onset of KD. But, early IVIG treatment could be a protective factor against the development of CAL, which needs to be further clarified.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

## INFORMED CONSENT

This systematic review and meta-analysis is based on a collection of data retrieved from studies that have already been published. We did not collect individual patient data and did not have direct contact with any of the included patients.

## AUTHOR CONTRIBUTIONS

FY, HZ, and FZ designed and conceived the experiments. FY and HZ performed the experiments. FY, RX, and XC analyzed the data. XC and YC contributed the reagents, materials, and analysis tools. FY, RX, and FZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.593435/full#supplementary-material

**Supplementary Figure 1 |** Sensitivity analysis of the included studies for the incidence analysis of CAL.

**Supplementary Figure 2 |** Sensitivity analysis of the included studies for the incidence analysis of IVIG unresponsiveness.

**Supplementary Figure 3 |** Pooled odds ratio for CAL development by the timing of IVIG therapy in KD (<5 days of disease onset vs. 5–10 days).

**Supplementary Table 1 |** The NEWCASTLE-OTTAWA SCALE for case control studies.

**Supplementary Table 2 |** The NEWCASTLE-OTTAWA SCALE for cohort studies.

**Supplementary Table 3 |** Meta-regression analysis of the included studies for the primary outcome (CAL development).

**Supplementary Table 4 |** Meta-regression analysis of the included studies for the primary outcome (IVIG unresponsiveness).

**Supplementary Checklist 1 |** PRISMA checklist.

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BRIEF RESEARCH REPORT
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# Comparison of Machine Learning Models for Prediction of Initial Intravenous Immunoglobulin Resistance in Children With Kawasaki Disease

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We constructed an optimal machine learning (ML) method for predicting intravenous immunoglobulin (IVIG) resistance in children with Kawasaki disease (KD) using commonly available clinical and laboratory variables. We retrospectively collected 98 clinical records of hospitalized children with KD (2–109 months of age). We found that 20 (20%) children were resistant to initial IVIG therapy. We trained three ML techniques, including logistic regression, linear support vector machine, and eXtreme gradient boosting with 10 variables against IVIG resistance. Moreover, we estimated the predictive performance based on nested 5-fold cross-validation (CV). We also selected variables using the recursive feature elimination method and performed the nested 5-fold CV with selected variables in a similar manner. We compared ML models with the existing system regardless of their predictive performance. Results of the area under the receiver operator characteristic curve were in the range of 0.58–0.60 in the all-variable model and 0.60–0.75 in the select model. The specificities were more than 0.90 and higher than those in existing scoring systems, but the sensitivities were lower. Three ML models based on demographics and routine laboratory variables did not provide reliable performance. This is possibly the first study that has attempted to establish a better predictive model. Additional biomarkers are probably needed to generate an effective prediction model.

**Keywords: area under the curve, extreme gradient boosting, support vector machine, logistic regression, nested cross-validation, predictive model**

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## INTRODUCTION

In developed countries, Kawasaki disease (KD) is the major cause of acquired heart disease in children (1). The main complication of KD is coronary artery abnormality (CAA) due to systemic vasculitis (1). The effectiveness of high-dose intravenous immunoglobulin (IVIG) therapy has been established as an initial KD treatment (2). However, approximately 10–20% children with KD are

refractory to this treatment and develop persistent or recurrent fever after initial IVIG therapy (3, 4). IVIG resistance is a risk factor for the occurrence of CAA (5). Moreover, the development of a more effective treatment options has been challenging. The American Heart Association has reported that patients who were predicted to be at a high risk for development of CAA may benefit from primary adjunctive therapy such as IVIG and corticosteroids (2). Therefore, developing a reliable tool for predicting IVIG resistance is important to reduce the occurrence of CAA.

Several scoring systems (6–12) have been proposed. However, the predictive capacity of the existing scoring systems may not be sufficient, and some scoring systems have poor predictive performance for external datasets (13–15). Machine learning (ML) techniques have been applied to clinical diagnosis and prognosis prediction in many fields of medicine (16). To the best of our knowledge, few studies have applied ML methods for predicting resistance to initial IVIG therapy in patients with KD (17). We aimed to construct an optimal ML method for predicting IVIG resistance in children with KD using commonly available clinical and laboratory variables.

# MATERIALS AND METHODS

## Patients and Data Collection

We retrospectively collected clinical records of patients with KD who were diagnosed based on the Japanese diagnostic guidelines for KD (18) and hospitalized at Tsugaruhoken Medical COOP Kensei Hospital between January 2010 and October 2019. Patients diagnosed with KD presented with minimum five of the six major symptoms, including fever. Patients with only four or less major symptoms and those with CAA were not included. We excluded children who received initial IVIG treatment $\geq$10 days after the onset and children administered initial doses of $<$2 g/kg/day. We defined the first illness day as the first day on which a patient had fever. We defined a responder as a patient whose temperature had decreased to $<37.5^{\circ}$C within 36 h after initial IVIG treatment (9, 15).

We collected the following data before the initial IVIG treatment: months of age, gender, illness days with IVIG administration, white blood cell count (WBC), neutrophil percentage, hematocrit (Ht), platelet count (PLT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBil), and sodium (Na), albumin (Alb), and C-reactive protein (CRP) levels. All these variables were available before treatment.

We defined coronary arteries as abnormal when the luminal diameters were more than 3.0 mm in children younger than 5 years or more than 4.0 mm in those 5 years and older, when the internal diameter of a segment was 1.5 times or greater than that of an adjacent segment, or when the luminal contour was evidently irregular (19). We recorded the maximum coronary artery diameter within 1 month after the onset of the disease.

## Statistical Analysis

We performed statistical analyses using Python version 3.6 (Python Software Foundation). We applied Mann–Whitney *U*-tests for continuous variables and Chi-square tests for categorical variables.

We evaluated the predictive performance of the three supervised ML classifiers and existing scoring systems. We trained logistic regression (LR) with L2 regularization, linear support vector machine (SVM), and eXtreme gradient boosting (XGB) models to predict IVIG resistance, using scikit-learn and XGBoost packages. We evaluated the predictive performance based on sensitivity, specificity, and area under the receiver operator characteristic curve (AUC). We produced three ML models with 10 variables that did not contain missing values (months of age, gender, illness days with IVIG administration, WBC, Ht, PLT, AST, Na, Alb, and CRP).

To evaluate the predictive performance of the three ML models and algorithms, we used the nested 5-fold cross-validation (CV) approach (20) with *GridSearchCV* for hyper-parameter optimization. We applied a nested CV procedure to estimate an unbiased generalization performance of ML algorithms (21). The two CV cycles included an inner loop for tuning hyper-parameters and outer loop for estimating performance in nested CV (**Figure 1**). First, the original dataset was divided into five data folds with approximately equal numbers of respondent and non-respondent cases. One data fold was reserved for *test fold*. The remaining four data folds (*training folds*) were passed to the inner loop. The inner loop performed 5-fold CVs to identify the best hyper-parameter combination. We selected the hyper-parameter combination that maximized each performance metrics over all steps of the inner loop. In LR and linear SVM models, the penalty parameter *C* was explored in [0.01, 0.1, 1, 10, and 100]. In XGB model, the maximum depth of a tree (max_depth), the minimum sum of instance weight needed in a child (min_child_weight), and gamma were explored in [3, 5], [1, 2, 3], and [0, 3, 10], respectively. For the other hyper-parameters, we used default values of the scikit-learn method. These were tuned by testing all possible hyper-parameter combinations in the inner CV. We then trained our model on *training folds* using the best hyper-parameter combination; thereafter, we evaluated model performance on the *test fold*. This process was repeated five times, once for each iteration of the outer loop. Finally, we calculated the average performance over 5-folds. We also repeated nested CVs 10 times in separate splits and derived the average of the results to avoid sampling bias and data overfitting.

Additionally, we selected variables using the recursive feature elimination method. Then, we performed a nested 5-fold CV with selected variables in a similar manner. In all, we have constructed

**Abbreviations:** Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the receiver operator characteristic curve; CAA, coronary artery abnormality; CRP, C-reactive protein; CV, cross-validation; Ht, hematocrit; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; LR, logistic regression; ML, machine learning; Na, sodium; PLT, platelet count; SVM, support vector machine; TBil, total bilirubin; WBC, white blood cell count; XGB, eXtreme gradient boosting.

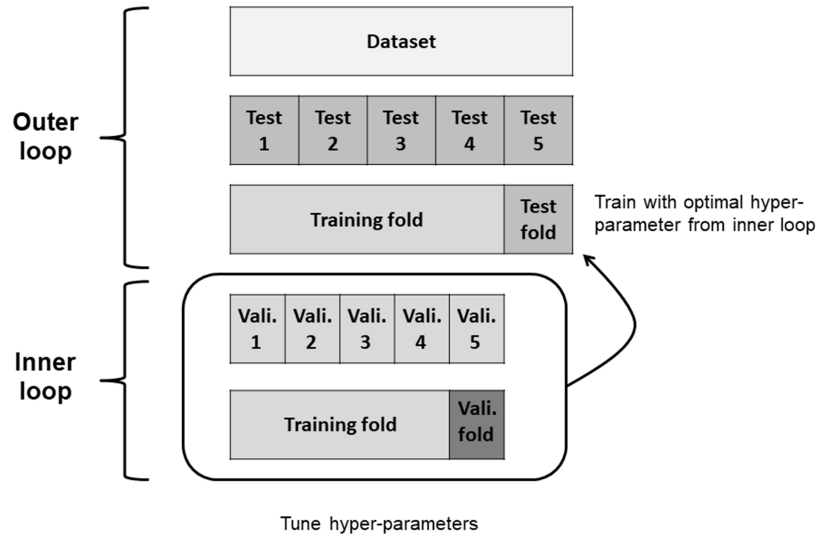


**FIGURE 1 |** Flow chart of the 5-fold nested cross-validation. Vali., validation.

and then evaluated two types of models: all-variable model and select-variable model.

# RESULTS

## Characteristics of Patients

We collected data from 109 children with KD treated at our hospital. We excluded data from 11 children because 9 children had received initial IVIG at <2 g/kg/day and 4 had received initial IVIG treatment ≥10 days after the onset of the disease. Consequently, we statistically analyzed data from 98 children aged 2–109 months. **Table 1** summarizes the demographic and laboratory data of patients. Among them, 20 (20%) children were resistant to the initial IVIG therapy. Only the AST and ALT levels were significantly higher in the IVIG-responsive group than in the IVIG resistant group. The proportion of CAA in the IVIG resistant group was higher than that in the IVIG-responsive group.

## Predictive Performance of the ML Model

As shown in **Table 2**, the AUCs of the all-variable models were 0.58–0.60 in all models, and those of the select-variable models were 0.60–0.75. The results on specificity and accuracy were 0.94–0.99 and 0.78–0.79 in the all-variable models, and 0.96–1.00 and 0.78–0.80 in the select-variable models. The results of specificity and accuracy were high, but those on sensitivity were all lower.

**TABLE 1 |** Comparison of clinical and laboratory characteristics in IVIG-responsive and -resistant patients.

| | Responsive ($n = 78$) | Resistant ($n = 20$) | *P*-value |
|---|---|---|---|
| Age, months of age, median (IQR) | 22 (9–37) | 26 (17–30) | 0.49 |
| Illness days with IVIG administration, days, median (IQR) | 5 (4–6) | 4 (3.8–5) | 0.16 |
| Gender, male, *n* (%) | 40 (51) | 13 (65) | 0.40 |
| White blood cell count, $\times 10^2/mm^3$, median (IQR) | 151 (121–175) | 144 (113–179) | 0.96 |
| Neutrophil, %, median (IQR) | 66 (59–76) | 73 (67–79) | 0.12 |
| Hematocrit, %, median (IQR) | 34 (32–36) | 35 (33–36) | 0.65 |
| Platelet count, $\times 10^4/mm^3$, median (IQR) | 35 (28–42) | 32 (27–38) | 0.41 |
| Aspartate aminotransferase, IU/L, median (IQR) | 30 (24–43) | 96 (34–308) | <0.001 |
| Alanine aminotransferase, IU/L, median (IQR) | 20 (12–32) | 75 (20–232) | 0.004 |
| Total bilirubin, mg/dl, median (IQR) | 0.53 (0.41–0.69) | 0.81 (0.50–1.37) | 0.36 |
| Sodium, mmol/L, median (IQR) | 133 (131–134) | 132 (131–134) | 0.88 |
| Albumin, g/dl, median (IQR) | 3.3 (3.1–3.6) | 3.4 (3.1–3.6) | 0.85 |
| C-reactive protein, mg/dl, median (IQR) | 6.3 (3.8–9.3) | 7.4 (5.3–10.6) | 0.26 |
| Coronary artery abnormalities, *n* (%) | 5 (6.4) | 6 (30) | ×0.007 |

*IVIG, intravenous immunoglobulin; SD, standard deviation; IQR; interquartile range. Data are analyzed by Mann–Whitney U tests for continuous variables and Chi-square tests for categorical variables.*

# DISCUSSION

We retrospectively evaluated the performances of three ML models to predict the resistance to initial IVIG therapy in a single-center pediatric population of KD. Our results revealed that the three ML models based on demographics and routine laboratory variables did not perform reliably.

Different clinical scoring systems have been established to predict IVIG resistance, including those by Kobayashi et al. (6), Egami et al. (7), and Sano et al. (8) in Japan. The sensitivities

**TABLE 2 |** Prediction performances of the three machine learning models and existing scoring systems.

| | | | Feature | AUC | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|---|---|---|
| All-variable model | LR | | All 10 variables | 0.59 ± 0.052 | 0.22 ± 0.055 | 0.94 ± 0.017 | 0.79 ± 0.021 |
| | Linear SVM | | All 10 variables | 0.58 ± 0.040 | 0.20 ± 0.059 | 0.95 ± 0.014 | 0.79 ± 0.018 |
| | XGBoost | | All 10 variables | 0.60 ± 0.048 | 0.26 ± 0.095 | 0.99 ± 0.021 | 0.78 ± 0.026 |
| Select-variable model | LR | Model 1 | AST | 0.75 ± 0.011 | 0.15 ± 0.039 | 0.97 ± 0.006 | 0.79 ± 0.012 |
| | | Model 2 | WBC, AST | 0.67 ± 0.027 | 0.16 ± 0.037 | 0.97 ± 0.008 | 0.80 ± 0.011 |
| | | Model 3 | Day, WBC, PLT, AST, CRP | 0.67 ± 0.022 | 0.19 ± 0.049 | 0.96 ± 0.049 | 0.80 ± 0.010 |
| | SVM | Model 1 | AST | 0.75 ± 0.011 | 0.14 ± 0.037 | 0.96 ± 0.012 | 0.79 ± 0.015 |
| | | Model 2 | WBC, Ht, PLT, AST | 0.66 ± 0.035 | 0.16 ± 0.039 | 0.97 ± 0.010 | 0.80 ± 0.010 |
| | | Model 3 | WBC, AST | 0.68 ± 0.024 | 0.14 ± 0.035 | 0.97 ± 0.006 | 0.79 ± 0.007 |
| | XGBoost | Model 1 | Na, AST | 0.65 ± 0.032 | 0.28 ± 0.078 | 1.00 ± 0.008 | 0.78 ± 0.021 |
| | | Model 2 | Age, Day, Ht, Na, AST, CRP | 0.61 ± 0.036 | 0.31 ± 0.073 | 0.99 ± 0.008 | 0.79 ± 0.025 |
| | | Model 3 | Age, Day, Ht, Na, AST, Alb, CRP | 0.60 ± 0.035 | 0.33 ± 0.078 | 0.99 ± 0.008 | 0.79 ± 0.022 |
| Existing scoring systems | Kobayashi (8) system ($n = 93$) | | | NA | 0.70 | 0.62 | 0.63 |
| | Egami (9) system ($n = 98$) | | | NA | 0.55 | 0.81 | 0.76 |
| | Sano (10) system ($n = 62$) | | | NA | 0.41 | 0.96 | 0.81 |

*AUC, area under the receiver operator characteristic curve; LR, logistic regression; SVM, support vector machine; XGB, eXtreme gradient boosting; Age, months of age; Day, illness days with IVIG administration; WBC, white blood cell count; Ht, hematocrit; PLT, platelet count; AST, aspartate aminotransferase; Na, sodium; Alb, albumin; CRP, C-reactive protein; NA, not applicable.*

and specificities of those systems were reported to be 0.76–0.78 and 0.76–0.86 in the original studies. However, almost all clinical scores published are limited in their predictive capacity. Similar predictive accuracies were not achieved in other populations (13–15). As shown in **Table 2**, the existing scoring systems also did not achieve a good prediction against our dataset.

Additional clinical information may be needed to improve the prediction model. Owing to the similarity of each clinical and laboratory characteristics between IVIG-responsive and -resistant patients in the current dataset, neither our model nor the existing model may have performed reliably. There may be a need to construct and evaluate new models that also incorporate clinical major symptoms (10) and/or other laboratory data such as erythrocyte sedimentation rate (10) or N-terminal pro-brain natriuretic peptide (22).

Our prediction models using three ML techniques have equally less reliable performance as the existing scoring systems; particularly, the sensitivity were low in all ML algorithms. Our results serve as a first step to establish a good prediction tool. Feature engineering or ensemble learning, which combines several ML techniques into one predictive model, may help improve performance. Alternatively, ML models have advantages over the existing prediction scoring systems. The predictive performances of scoring systems could differ depending on countries or ethnicities (11, 13, 23). ML is flexible and can be suitable for many tasks. Therefore, the ML approach makes it easy for the model to retrain and update the using the newest data.

To our best knowledge, this is the first study to compare the performances of ML methods for predicting IVIG resistance. There is a study which was designed to develop the prediction model using random forest (17). However, validation procedures were not conducted, though the performance was excellent. Conversely, there are certain limitations. First, the dataset was relatively small. However, we used nested CVs to obtain unbiased estimates of the true error. We also repeated the nested CVs 10 times and averaged the validation error to reduce sampling bias. Nested CV can choose the classification model by obtaining reliable classification performance and avoiding overfitting (24). Second, the present study was conducted based on dataset derived from a single center. Accordingly, our results may not apply to other populations. However, we consider it meaningful to rebuild the model, similarly using the center's original data. Third, this is a retrospective study. We need to perform a combined analysis of three ML models on a prospective basis.

In conclusion, we evaluated the performance of ML models for predicting resistance to IVIG therapy in children with KD. However, our three ML models based only on demographics and routine laboratory variables did not provide reliable performances. Further studies are needed to improve predictive models. Additional biomarkers are likely to be needed to generate an effective prediction model.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, Yasutaka Kuniyoshi, upon reasonable request.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Tsugaruhoken Medial COOP Kensei Hospital. Written informed consent from the participants' legal guardian/next of kin was not required to participate in

this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

YK designed the study, drafted the manuscript, performed the statistical analysis, and interpreted the results. All authors have read and approved the final manuscript and contributed to data collection.

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# Brainstem Auditory Evoked Potentials and Visual Potentials in Kawasaki Disease: An Observational Monocentric Study

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**Background:** Kawasaki Disease is a systemic vasculitis, particularly involving coronary arteries. Rare involvement of other vascular districts is described, as central nervous system arteries, leading to a vasculitic neuropathy. Sensorineural hearing loss and alterations of evoked potentials are uncommonly reported complications.

**Methods:** In an observational monocentric study, 59 children (37 males; 22 females; mean age: 2.7 ± 2.2 years) with documented Kawasaki Disease were enrolled. No risk factors for hearing loss and/or neurological impairment were identified in the cohort. Brainstem auditory evoked potentials and visual evoked potentials were correlated with clinical, hamatological and radiological data, evaluated in the acute phase of the Kawasaki Disease, and during the follow-up.

**Results:** Evoked potentials were altered in 39/59 patients (66%): of these, 27/39 (69%) showed altered IV and V waves and/or III-V interwave latencies of brainstem auditory evoked potentials; 4/39 (10%) showed pathological visual evoked potentials; 8/39 (21%) had abnormalities of both brainstem auditory evoked potentials and visual evoked potentials. No permanent deafness was reported.

**Conclusion:** Abnormalities in visual evoked potentials were not significantly correlated with coronary artery lesions; however, the presence of abnormalities of brainstem auditory evoked potentials were associated with the risk of coronary artery lesions.

**Keywords: kawasaki disease, brainstem auditory evoked potentials, visual evoked potentials, coronary artery lesions, intravenous immunoglobulins**

## INTRODUCTION

Coronary artery lesions (CAL) and/or cardiac lesions are the fearsome evolution of Kawasaki disease (KD). Nevertheless, other vascular districts may be involved less frequently, such as central nervous system (CNS) arteries (1, 2), peripheral *vasa nervorum* and perineural blood vessels, leading to a vasculitic neuropathy of acoustic nerve and optic nerve (3, 4).

A severe cochlear or vessel wall inflammation can induce hearing loss and/or subclinical alteration of brainstem auditory evoked potentials (BAEPs). Optic nerve could be involved as well, with a pathological pattern of visual evoked potentials (VEPs). However. these abnormalities are sporadic and have been described just in a few reports (4, 5).

In the literature, only few studies focused on BAEPs and VEPs in KD (6). There are also some case reports of auditory loss secondary to KD (4, 5).

Sensorineural hearing loss may be underreported, since a reduced hearing acuity may be only transient and since when the condition is present in young children it could not be promptly recognized. Hearing loss is often detected only by audiometry or, in young children in whom tone audiometry is difficult to be carried out, by BAEPs.

Some reports showed that up a third of KD children had a sensorineural auditory loss during the acute and subacute phases of the disease, and that the auditory loss could be maintained even six months after diagnosis (7, 8). The authors observed a positive association of this complication with anemia and thrombocytosis. VEPs and BAEPs may be pathological in the acute phase of KD, however they can normalize during the follow-up. This finding may be the sign of systemic vasculitis, involving CNS.

CAL may have poor correlation with clinical signs; however, hematological parameters, expression of critical cytokine secretion (such as leucocytosis, increased neutrophil percentage, elevation of CRP, increased transaminases, hypoalbuminemia and hyponatremia), are correlated with a more severe evolution and an increased risk to develop CAL. Pharmacological approach of KD with IVIG within 7-10 days since KD onset is efficacious to prevent CAL. However, there are no data about hematological parameters and the treatment choice on the prevention of evoked potentials alterations.

A systemic vasculitis could involve many districts at the same time and in the acute phase of the disease; the synchronous involvement of coronaries and CNS districts in KD has not yet been demonstrated.

## Aims of the Study

Aims of the study were:

- the evaluation of VEPs and BAEPs in children affected by KD, in the acute phase of the disease and during the follow-up;
- the evaluation of the role of evoked potentials as an added tool to detect occurrence of vasculitis in the anatomic and functional systems respectively explored by VEPs and BAEPs;
- the correlation of VEPs and BAEPs with clinical signs, hematological parameters, treatment with IVIG, aspirin and other drugs;
- the correlation between CAL and/or cardiac lesions, and dysfunction of VEPs and BAEPs at diagnosis and during the follow up.

**Abbreviations:** KD, Kawasaki Disease; BAEPs, brainstem auditory evoked potentials; VEPs, visual evoked potentials; CAL, coronary artery lesions; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate.

# MATERIALS AND METHODS

## Patients

In an observational monocentric study, we enrolled 59 children (37 M; 22 F; mean age: 2.7 ± 2.2 years; range 3 months−10 years) with documented KD. All the patients, followed from 2012 to 2018 in the Pediatric Clinic of Palermo, Children Hospital "G. Di Cristina," ARNAS Palermo, with a good compliance to the procedures of evaluation of evoked potentials, and no risk factors for hearing loss and/or neurological impairment (CNS congenital diseases, developmental delay, ASA intoxication, etc.) were included. Of those, 37 children (63%) had typical KD, eight patients (14%) atypical KD, and 14 patients (23%) an incomplete form. All patients were treated with 2 g/kg of intravenous immunoglobulins (IVIG) and acetylsalicylic acid (ASA) at the dosage of 30–50 mg/kg/day in the acute phase, and 3–5 mg/kg /day as antiplatelet prophylaxis. BAEPs and VEPs were correlated with clinical, hematological and radiological data, evaluated in the acute phase of the KD, and during the follow up.

## Clinical and Demographic Data

Age, gender, days of illness at initial treatment and response to IVIG and ASA; rash; changes of lips and oral mucosa; conjunctivitis; cervical lymphadenopathy.

## Hematological Parameters

White blood cell count, neutrophils percentage, platelet count, hemoglobin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), transaminases, gamma-glutamyl transferase, ferritin, albumin, sodium, D-dimer.

## Imaging Data

Chest x-ray, echocardiogram, abdominal ultrasound.

## Diagnostic Criteria

KD was diagnosed following the internationally approved criteria (9, 10):

A. Typical KD was diagnosed when fever lasting more than 5 days was associated with ≥4 clinical criteria:
   1. bilateral non-exudative conjunctivitis
   2. changes of oral mucosa and lips
   3. changes of the extremities
   4. polymorphous exanthema
   5. cervical lymphadenopathy.

B. Incomplete KD was suggested in every infant showing fever lasting more than 5 days with documented systemic inflammation if <4 main clinical features were found after exclusion of many febrile illnesses.

C. Atypical KD was diagnosed if fever lasting more than 5 days, not otherwise explained, was associated with non-classic manifestations (as sensorineural hearing loss, aseptic meningitis, seizures, peripheral facial nerve palsy, acute abdomen, pancreatitis, gallbladder hydrops, pneumonia, arthritis, orchitis, renal impairmant, sterile pyuria). CAL documented by echocardiography confirmed the diagnosis.

## Ethics and Ethics Committee

Ethics committee approval according to local regulations was not necessary since this was part of our clinical practice and since deidentified data were used for statistical analysis. However, the study involving human participants was reviewed and approved by the ethics committee Palermo 1 (ARNAS Civico, Palermo, Italy). The patients' legal guardian provided written informed consent to participate in this study. The written consent is in the documents in the hospital "G. Di Cristina", ARNAS Palermo, Italy.

## Methods

VEPs and BAEPs were recorded by a MYOQUICK SystemPLUS Evolution (Micromed, Italian). The relevant recording techniques are described hereunder.

The latencies evaluation of waves I, III and V were performed in all patients together with the amplitudes of waves I and V. Furthermore, the V/I amplitude ratio, and the I-III and III-V interwave latencies were calculated. Recorded abnormalities consisted of decreased amplitude of wave V and prolonged III-V interpeak latencies.

The recordings were collected in the acute phase of the disease, in the few days (1–7, 9–11) after defervescence occurred, before discharge from the hospital, and during follow-up (6 and 12 months after discharge).

## VEPs

VEPs were recorded from an active midline occipital electrode over the visual cortex at $O_z$, with a midline frontal reference electrode at $F_z$, according to the Recommendations and Guidelines of the International Federation of Clinical Neurophysiology (IFCN) Committee (12) and International Society for Clinical Electrophysiology of Vision (ISCEV) (13). On the basis of children's age and of his/her degree of cooperation during the exam, different types of stimulus have been used.

**Flash VEPs** were performed with monocular stimulation. The unstimulated eye was occluded to avoid extraneous and unwanted stimulation. The potentials were carried out using a flash (brief luminance increment) with a stimulation force of 3 cd s $m^{-2}$ (photopic candles seconds per square meter) which subtends a visual field of at least 20° in a dimly illuminated room.

- **Pattern Reversal Visual Evoked Potentials (PRVEPs)** were performed with monocular stimulation and full-field stimulation.

The standard stimulus for VEPs is a checkerboard model in which the squares take turns from black to white: the dark squares become light and vice versa without changing the general luminance of the display.

The pattern was reversed 100 times at 1 Hz and the results were then averaged; A repeated trial of averaged stimuli was also recorded.

## BAEPs

The BAEPS were carried out and interpreted according to the guidelines of the American Clinical Neurophysiology Society (ACNS) (14).

BAEPs were recorded from an active electrode positioned over Cz and referred to the right (M2) and to the left (M1) mastoid process. Waveforms are recorded from ipsilateral and contralateral pathways simultaneously, allowing easier recognition of individual peaks.

The following regards others technical details:

- the stimuli consisted of 100/microseconds clicks delivered to each ear in turn through pre-calibrated shielded earphones at a rate of 10.6 Hz.
- the stimulus intensity was 60 dB above the hearing threshold (14) for each individual ear with the contralateral ear masked by white noise of 40 dB below the stimulus intensity;
- Thousand and five hundred or more stimuli, depending on the shape and amplitude of the potentials, were averaged;
- the latencies of waves I, III, and V were measured together with the amplitudes of waves I and V. The V/I amplitude ratio, and the I-III and III-V interweaves latencies were also calculated.

## Statistics

Patients were classified by gender, age, treatment duration and evoked potentials normal or altered. Correlation between the analyzed parameters collected were performed using Chi-square test. All variables were tested for normal distribution using the Anderson-Darling normality test. All ordinal data were expressed as numbers and percentages. Calculations were done using "MiniTAB release 13.1 Statistical Software."

# RESULTS

## Evoked Potentials Abnormalities

The data of evoked potentials findings for all the patients, divided in three groups of KD (typical, atypical, incomplete), are reported in **Table 1**.

Evoked potentials were altered in 39/59 patients (66%) (26 M; 13 F): among these, 27/39 (69%) (18 M; 9 F) had pathological BAEPs; 4/39 (10%) (2 M; 2 F) had pathological VEPs; 8/39 (21%) (6 M; 2 F) had abnormalities of both BAEPs and VEPs. Hence, BAEPs were altered in 35 patients (24 M; 11 F) (**Table 2**).

Pathological BAEPs waves were documented as bilateral in most of the patients. However, 12 patients (31%) showed unilateral pathological BAEPs.

In two patients hearing loss was documented, and one of them showed neurosensorial hypoacusis. This was a 4 month-old baby, who had complete KD with CAL, in whom hypoacusis was found after the resolution of the acute phase and was associated with pathological VEPs: at the following controls, VEPs were still pathological, while BAEPs were in the normal range and hearing loss had resolved. He was treated with IVIG 8 days after KD onset and did not receive steroids.

In the patients with VEP abnormalities, the prevalent recorded features were the increased latency of P100 /P2 wave-Flash VEP (P2) or the pattern VEP (P100).

There was no significant difference in incidence of evoked potentials alterations between typical or incomplete KD patients.

**TABLE 1 |** BAEPs and VEPs findings (normal or pathological) were divided in three groups of KD (typical, atypical and incomplete KD).

| | **Patients *n* = 59** | **Normal BAEPs and VEPs 20/59 (34%)** | **Pathological BAEPs only 27/59 (45%)** | **Pathological VEPs only 4/59 (7%)** | **Pathological BAEPs and VEPs 8/59 (14%)** |
|---|---|---|---|---|---|
| Typical KD | 37/59 (63%) | 11/37 (30%) | 19/37 (51%) | 1/37 (3%) | 6/37 (16%) |
| Atypical KD | 8/59 (14%) | 4/8 (50%) | 2/8 (25%) | 2/8 (25%) | 0 |
| Incomplete KD | 14/59 (23%) | 5/14 (36%) | 6/14 (43%) | 1/14 (7%) | 2/14 (14%) |

**TABLE 2 |** BAEPs and VEPs findings in KD patients: 26/37 (70%) of males and 13/22 (59%) of females showed pathological evoked potentials.

| | **Patients with pathological evoked potentials *n* = 39** | **Pathological BAEPs only 27/39 (69%)** | **Pathological VEPs only 4/39 (10%)** | **Pathological BAEPs and VEPs 8/39 (21%)** |
|---|---|---|---|---|
| Males (37) | 26 (67%) | 18 (67%) | 2 (50%) | 6 (75%) |
| Females (22) | 13 (33%) | 9 (33%) | 2 (50%) | 2 (25%) |

**TABLE 3 |** Pathological BAEPs and VEPs and CAL: distribution of the cases.

| | **Patients *n* = 59** | **Normal BAEPs and VEPs 20/59 (34%)** | **Pathological BAEPs only 27/59 (46%)** | **Pathological VEPs only 4/59 (7%)** | **Pathological BAEPs and VEPs 8/59 (14%)** |
|---|---|---|---|---|---|
| No CAL | 42 (71%) | 14/42 (33%) | 21/42 (50%) | 3/42 (7%) | 4/42 (10%) |
| CAL | 17 (29%) | 6/17 (35%) | 6/17 (35%) | 1/17 (6%) | 4/17 (24%) |

## Cardiac Involvement

In our patients, CAL were detected in 17/59 patients (29%): six children (10%) showed coronaritis without aneurysms, 11 (19%) developed aneurysms. 6/17 children with CAL had normal BAEPs and VEPs; 4/17 children showed both altered BAEPs and VEPs; 6/17 patients had altered BAEPs; 1/17 patients had altered VEPs (**Table 3**).

The incidence of pathological BAEPs and VEPs was higher than CAL; in fact, 71% of the patients had normal echocardiogram, while only 34% of the patients showed normal evoked potentials. Nevertheless, 50% of children with both pathological BAEPs and VEPs had CAL; 35% of patients with pathological BAEPs and normal VEPs showed CAL; 6% of patients with pathological VEPs and normal BAEPs had CAL.

In some patients of our case series, evoked potentials alterations appeared earlier than CAL, while in two patients these alterations were detected only during follow-up (at 12 months after disease onset) (**Table 4**).

## Correlation With Clinical and Hematologic Parameters

No correlation was seen between BAEPs and VEPs alterations and days of fever at the start of IVIG, pre-IVIG D-Dimer plasma levels, CRP, ESR, leukocyte count, neutrophil percentage pre- and post- IVIG, number of IVIG doses.

**TABLE 4 |** BAEPs and VEPs during the acute phase and 12 months after the onset of the disease.

| **Patients with pathological evoked potentials** | **Patients with pathological evoked potentials normalized during the follow-up** | **Patients with normal evoked potentials pathological during the follow-up** |
|---|---|---|
| 39 | 3 | 2 |

## Correlation With Treatment Strategies

Furthermore, pathological evoked potentials and the resolution of these abnormalities in 3/39 (8%) patients during follow-up, were not correlated with corticosteroid treatment (which was administered in eight patients), while treatment with anakinra induced the resolution of CAL, as documented in the literature (15, 16) and the normalization of evoked potentials in one child (**Figure 1**).

# DISCUSSION

The patients studied in our series showed altered IV, V waves and/or III-V interwave latencies, despite normal hearing acuity. These children showed a possible CNS vasculitis, and in a high percentage of them CAL were also present.

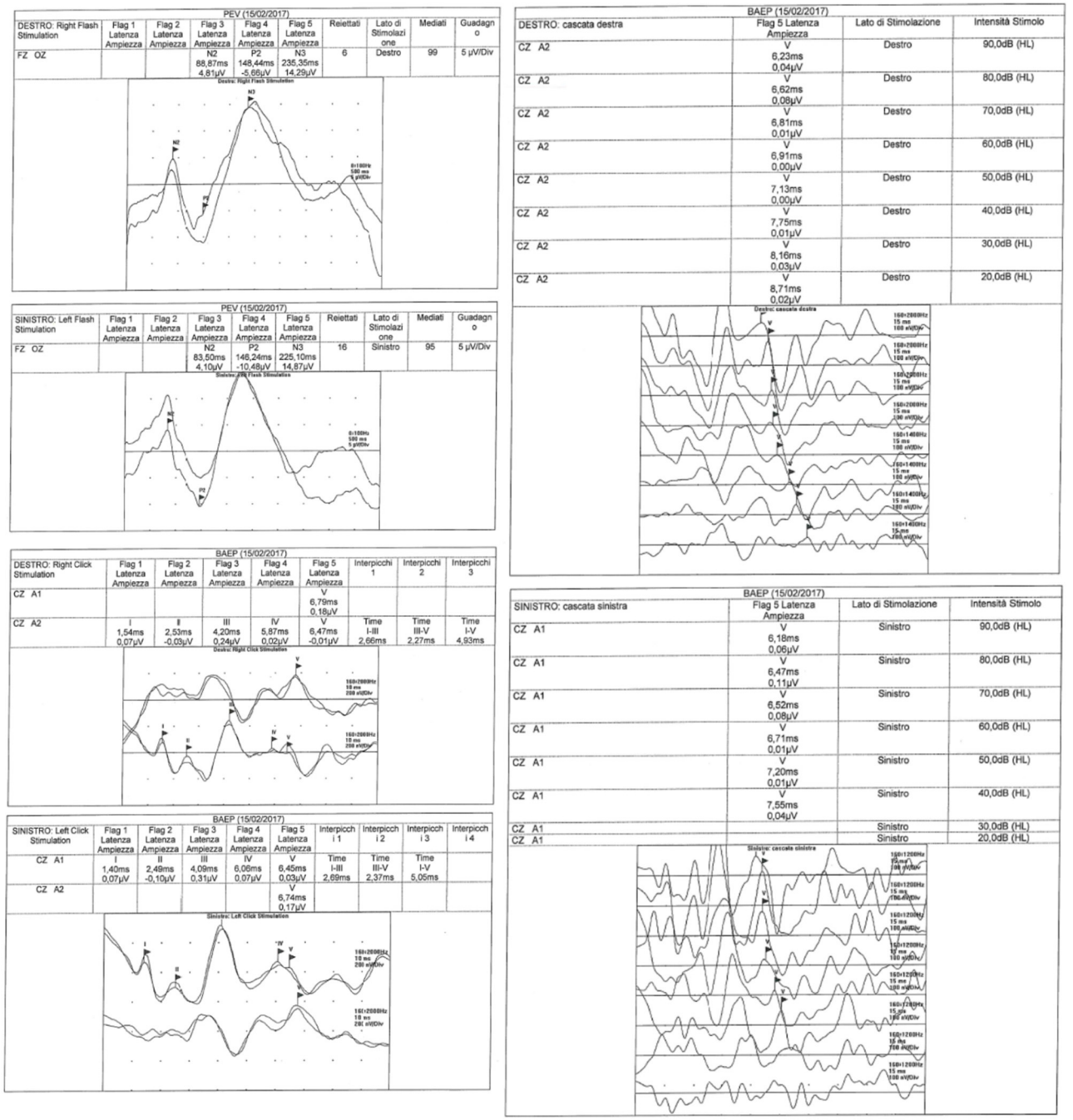


**FIGURE 1 |** VEPs and BAEPs in a patient with pathological records.

Recorded abnormalities of BAEPs consisted of decreased amplitude of wave V, prolonged III-V interpeak latencies caused by slowed conduction within the lower pons and the midbrain (17).

In this case series evoked potentials were pathological in a high number of patients: 70% of males and 59% of females. BAEPs, alone or in association with VEPs, were more frequently pathological than VEPs alone. In fact, isolated alteration of VEPs was found in only 7% of the patients. Otherwise, isolated alterations of BAEPs were detected in 46% of the patients, and most of them showed alterations secondary to mesencephalic involvement.

Despite normal hearing acuity in almost all cases, the patients of our cohort showed a neuronal involvement, documented by evoked potentials. The dysfunction of our patients seemed secondary to a mesencephalic damage. This pattern could be

an expression of a vasculitis of CNS, which has already been documented in KD children in other encephalic districts (1, 2, 8). In 43% of these children CAL were present, possibly as further expression of systemic vasculitis.

The abnormalities of BAEPs and VEPs are not necessarily linked to the acute phase of KD; in fact, these alterations have been documented also months after KD onset, and some patients may maintain a permanent loss of function with an auditory loss (3, 5, 7, 18–20). The spectrum of complications in KD includes not only CAL and other cardiac lesions, but also CNS involvement.

In our study, the percentage of children with pathological BAEPs and/or VEPs in association with CAL is high. However, the differences between the groups of patients with specific evoked potentials alterations did not reach the statistical significance, but the number of patients in the specific groups are small and need further confirmation in a larger population sample. Moreover, our study of CNS involvement was performed by evoked potentials, suggesting a functional involvement of the encephalic districts, but was not confirmed by Magnetic Resonance Angiography. Further studies could be integrated with Magnetic Resonance Angiography in children with KD, to document anatomic damage secondary to systemic vasculitis.

A multicentre report on KD patients showed transient sensorineural hearing loss as a possible complication of acute KD; this event was considered secondary to salicylate toxicity (21). A persistent sensorineural hearing loss is rare (19). High doses of salicylates could cause severe hearing loss; however, in our series all patients were treated with low-medium doses of ASA. Case reports described bilateral severe sensorineural hearing loss, in some cases treated with corticosteroids; some experienced only a partial improvement, with possible severe persisting hearing loss (18, 19).

An association between persistent sensorineural hearing loss and persistent thrombocytosis, anemia, high ESR and late administration of IVIG has been described (7); in our case series this association was not found, neither with hematological parameters nor with clinical signs. Furthermore, IVIG, steroids and ASA timing of administration was not correlated with evoked potentials alterations. Hence, the finding of evoked potentials abnormalities did not modify the treatment of our patients; however, they can help the clinicians in the diagnosis, especially in atypical or incomplete cases. In this regard, treatment can be guided by BAEPs and VEPs study, in terms of an earlier diagnostic suspicion and a prompt therapeutic choice.

A generalized vasculitis, involving CNS vessels, was documented in KD (2, 22), describing hypoperfusion events that occur in the acute phase of KD, and provides another physio-pathological hypothesis that supports potentials alteration. In fact, as further confirmation of the hypothesis of the systemic vasculitis, in our series several patients showed CAL as well. We could not demonstrate a statistically significant association between abnormal potential and CAL, but the limited number of children of each group did not contribute to achieve a statistically significant difference.

## CONCLUSION

In conclusion, sensorineural hearing loss and alterations of evoked potentials are uncommonly reported complications of KD. In the present sample of patients, CNS involvement was always subclinical, without symptoms otherwise reported by patients and/or parents. These data alert the clinicians about the meaning of pre-clinical evoked potentials abnormalities in the management of KD patients. We hypothesize that vasculitis of vasa nervorum is a possible pathogenetic event linked to evoked potentials alterations. Evoked potentials allow to detect brain dysfunction, supporting brain involvement and precociously helping to identify permanent lesions that can induce disability if underdiagnosed. Furthermore, the diagnosis of atypical or incomplete KD is further supported by evoked potentials study.

A few studies proposed that asymptomatic cerebral vasculitis might be more frequent than expected on the basis of clinical signs (23). Therefore, pediatricians must consider the possible vascular involvement beyond CAL in KD, especially in the cerebral vessels, for the life-treating consequences and the poor prognosis of these patients.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The study involving human participants was reviewed and approved by the ethics committee Palermo 1 (ARNAS Civico, Palermo, Italy. The patients' legal guardian provided written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

MM, GS, and DP: substantial contributions to conception and design, acquisition of the data, analysis and interpretation of the data. RC and GC: drafted the article and revised it critically for important intellectual content. MM, GC, GS, DP, and RC: final approval of the version to be published. All named authors have agreed to its submission.

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# Prevalence and Clinical Characteristics of SARS-CoV-2 Confirmed and Negative Kawasaki Disease Patients During the Pandemic in Spain

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**Introduction:** COVID-19 has a less severe course in children. In April 2020, some children presented with signs of multisystem inflammation with clinical signs overlapping with Kawasaki disease (KD), most of them requiring admission to the pediatric intensive care unit (PICU). This study aimed to describe the prevalence and clinical characteristics of KD SARS-CoV-2 confirmed and negative patients during the pandemic in Spain.

**Material and Methods:** Medical data of KD patients from January 1, 2018 until May 30, 2020 was collected from the KAWA-RACE study group. We compared the KD cases diagnosed during the COVID-19 period (March 1–May 30, 2020) that were either SARS-CoV-2 confirmed (CoV+) or negative (CoV–) to those from the same period during 2018 and 2019 (PreCoV).

**Results:** One hundred and twenty-four cases were collected. There was a significant increase in cases and PICU admissions in 2020 ($P$-trend = 0.001 and 0.0004, respectively). CoV+ patients were significantly older (7.5 vs. 2.5 yr) and mainly non-Caucasian (64 vs. 29%), had incomplete KD presentation (73 vs. 32%), lower leucocyte (9.5 vs. 15.5 $\times$ $10^9$) and platelet count (174 vs. 423 $\times$ $10^9$/L), higher inflammatory markers (C-Reactive Protein 18.5vs. 10.9 mg/dl)

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and terminal segment of the natriuretic atrial peptide (4,766 vs. 505 pg/ml), less aneurysm development (3.8 vs. 11.1%), and more myocardial dysfunction (30.8 vs. 1.6%) than PreCoV patients. Respiratory symptoms were not increased during the COVID-19 period.

**Conclusion:** The KD CoV+ patients mostly meet pediatric inflammatory multisystem syndrome temporally associated with COVID-19/multisystem inflammatory syndrome in children criteria. Whether this is a novel entity or the same disease on different ends of the spectrum is yet to be clarified.

**Keywords: Kawasaki disease (KD), children, SARS-CoV-2, COVID-19, shock, multisystem inflammatory syndrome in children (MIS-C), pediatric inflammatory multisystem syndrome (PIMS-TS)**

## INTRODUCTION

The epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing COVID-19, has spread rapidly around the globe (1). Spain was one of the first European countries to be affected after Italy, with the outbreak estimated to have started in February 2020.

In contrast with adults, the disease in children appears to have a less severe course, with almost no fatalities, and those reported were mainly in children with severe underlining conditions (2, 3). But in April, some children presented critically ill with fever, shock, and signs of multisystem inflammation most of them requiring admission to the pediatric intensive care unit (PICU). They presented clinical signs overlapping with Kawasaki disease (KD) (4, 5) triggering alerts to pediatricians. Following the alert, the World Health Organization (WHO) (6), the European Centre for Disease Prevention and Control (7), the US Centers for Disease Control and Prevention (8), and the Royal Collage of Paediatrics and Child Health (9) have all produced definitions for this new entity. It was called pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 infection (PIMS-TS) (9) or multisystem inflammatory syndrome in children (MIS-C) (8) and case definition and guidance on clinical management was published.

The cause of KD remains unknown; however, it is suggested that an infectious agent might trigger the illness (10). A small proportion of KD patients present with Kawasaki disease shock syndrome (KDSS) resembling PIMS-TS/MIS-C (11). Cases of KD with concurrent COVID-19 infection were reported (12) suggesting that SARS-CoV-2 may trigger a cytokine storm leading to this newly defined syndrome (13–16).

This study aimed to compare the prevalence and features of KD patients before SARS-CoV-2 pandemic and compare them to the SARS-CoV-2 positive and negative cases presenting during the pandemic.

## MATERIALS AND METHODS

### Network Setup

During 2015 a nationwide KD study group named KAWA-RACE was setup. Patients were included retrospectively from 2011 through 2016 and prospectively from 2018 onward, based on declaration from pediatricians of recruiting centers. A total of 93 Spanish hospitals joined the network. Our study complies with the Declaration of Helsinki and the ethics committee at Instituto de Investigación Hospital 12 de Octubre approved this study (CEIC 15/316). The inclusion of patients with KD was approved following informed consent from parents/guardians. All patient data were fully anonymized before we accessed them, and then the database was reviewed to clean inconsistencies and confirm patients' diagnoses based on information provided.

### Data Source, Collection, and Management

Prospective medical data was included from January 1, 2018 until May 30, 2020. A research electronic database capture (17) was created and sent to the participant clinicians together with the study protocol.

We established the date of March 1, 2020 as SARS-CoV-2 exposed cases, collected cases until May 30, 2020, and considered this COVID-19 period (CoV-19p). The patients' demographic, clinical, laboratory, and echocardiographic data were recorded.

### Subjects and Case Definitions

Individual patient data were reviewed to confirm the diagnosis of complete or incomplete KD according to the American Heart Association (AHA, 2017) (18). Coronary arteries measurements followed Z-score of Montreal scale (19), and the classification according to the Z-score followed AHA guidelines (18); coronary artery lesions (CAL) were considered if the Z-score $> 2$, and cardiac dysfunction was considered if ejection fraction was $<55\%$ (20). All patients $<16$ years of age diagnosed with KD were included in the study. We looked at the SARS-CoV-2-positive patients to see if they fulfilled the WHO definition of MIS-C (8) and/or the Royal College of Pediatrics and Child Health definition of PIMS-TS (9).

Two groups were established for comparison. We compared the KD cases diagnosed during the same period of 2018 and 2019 (PreCoV) to those during CoV-19p that were SARS-CoV-2 negative confirmed by both polymerase chain reaction (PCR) and serology (CoV-) and to those during CoV-19p that were SARS-CoV-2 confirmed with either PCR and/or serology (CoV+).

To assess the prevalence, we compared the same periods of 2018, 2019, and 2020 (March 1, 2020–May 30, 2020). We excluded patients older than 16 years at the time of diagnosis, those patients diagnosed from January 1 to February 29, 2020, because the virus could be circulating undetected, but tests were not routinely performed and patients in whom PCR and serology

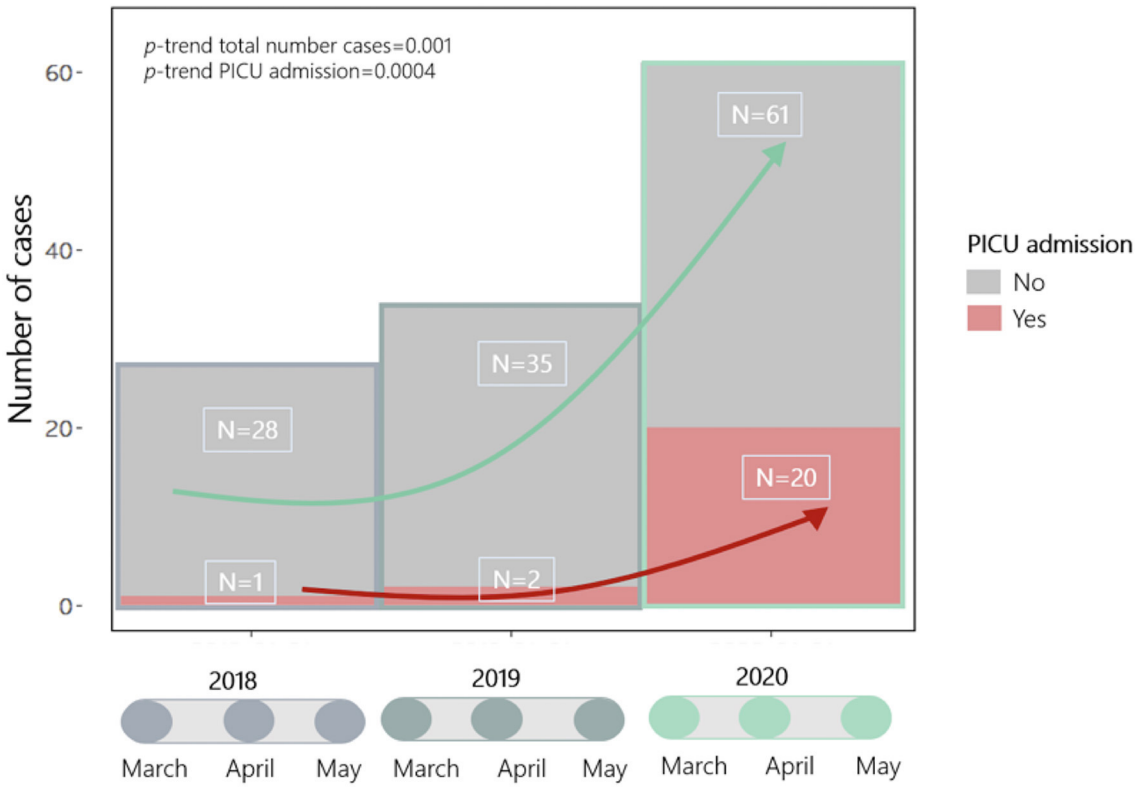


**FIGURE 1 |** Trend in Kawasaki disease cases and PICU admissions from 1st March to 30th May during 2018–19 (preCoV-19p) and 2020 (CoV-19p).

were not performed. Therefore, we could not assure they were SARS-CoV-2 negative.

## Statistical Analysis

Baseline characteristics were described through summary tables reporting frequencies and total records in case of categorical variables and median [interquartile range (IQR)] when continuous. Chi-squared and Fisher-test (low cell sizes $< 5$) were applied to assess differences among periods for categorical variables. For continuous variables, the non-parametric U-Mann-Whitney test was applied. Normality was tested with the Shapiro-Wilk test. The incidence was estimated using the incidence R package (21), and trend was calculated using the Chi-squared test. R software was used for all analysis (22).

# RESULTS

Nationwide, 124 cases were collected during the periods March 1–May 30, 2018/19/20 with 23 (19%) requiring PICU; the diagnosis of KD was confirmed for all patients after individual data review. There was a significant increase in cases and PICU admissions in 2020 ($P$-trend = 0.001 and 0.0004, respectively) (**Figure 1**).

For comparison with the CoV– and CoV+ groups, we excluded 15 patients in whom PCR and serology were not performed, and therefore we could not assure they were SARS-CoV-2 negative, leaving 109 patients included for further analysis—63 in PreCoV group, 26 CoV+, and 20 CoV–. In the CoV+ group, SARS-CoV-2 PCR was positive in 42% (11/26); SARS-CoV-2 serology was positive in 100% (21/21) of patients that had the test performed, and both were positive in 29% ($n =$ 6). All the patients in the CoV- group had a SARS-CoV-2 PCR and serology performed that were both negative (**Tables 1**, **2**).

## Clinical Characteristics (Table 3)

The median PreCoV age was 2.55 (IQR, 1.5–3.9), 3.56 (IQR, 2.2–6.4) for CoV– and significantly higher 7.54 (IQR, 5.4–10.8) for CoV+ patients ($p < 001$). There was a male predominance PreCoV (62%) and in CoV+ (69%) with a 1:1 ratio in CoV- patients. We found that in PreCoV and CoV– patients, around one-fourth of them were non-Caucasian (18/63, 29%, and 5/20, 25%, respectively), while in CoV+ patients this was significantly higher (16/26, 64%). The median duration of fever was 7 days in all groups.

Complete KD was less frequent during the CoV-19p [(CoV– 8/20, 40%) and (CoV+ 7/26, 27%) vs. PreCoV (43/63, 68%)]. Of note during the CoV-19p, especially in the CoV+ group, up to 23% of patients were suspected and treated as KD but did not fulfill AHA criteria (complete or incomplete), while during the PreCoV this did not happen. Patients from CoV-19p who did

**TABLE 1 |** Microbiological SARS-CoV-2 and blood test results of patients with Kawasaki disease (2018–2019) vs 2020 (*CoV+) and (CoV-)*.

| | **2018–2019 *N* = 63** | **2020 (*CoV+*) *n* = 26** | ***p*. value** | **2020 (*CoV-*) *N* = 20** | ***p*. value[#]** |
|---|---|---|---|---|---|
| **Microbiological Results** | | | | | |
| Positive result (non-SARS-CoV-2) | 17 (27%) | 3 (15%) | 0.227 | 4 (20.0%) | 0.659 |
| Coronavirus PCR performed | – | 26 (100%) | | 20 (100%) | |
| Positive | – | 11 (42.3%) | | 0 (0.00%) | |
| Coronavirus Serology performed | – | 21 (80.8%) | | 20 (100%) | |
| Positive | – | 21 (100%)* | | 0 (0.00%) | |
| PCR+ and Serology+ | – | 6 (28.6%) | | 0 (0.00%) | |
| **Blood tests** | | | | | |
| Hemoglobin (g/dL) | 11.6 (10.6; 12.2) | 12.2 (11.0; 13.3) | 0.018 | 10.9 (10.3; 11.9) | 0.547 |
| Leucocytes $\times 10^9$ /L | 15.5 (11.9; 20.1) | 9.50 (7.40; 12.8) | < 0.001 | 13.8 (8.98; 21.7) | 0.434 |
| Platelets at admission | 423 (301; 538) | 174 (118; 250) | < 0.001 | 294 (232; 423) | 0.024 |
| Platelets (min) | 371 (284; 446) | 174 (118; 250) | < 0.001 | 290 (193; 423) | 0.125 |
| Platelets (max) | 605 (530; 735) | 542 (394; 703) | 0.064 | 599 (299; 711) | 0.225 |
| ESR (mm/h) | 67.0 (56.5; 86.5) | 46.0 (11.5; 61.0) | 0.010 | 56.0 (33.2; 72.0) | 0.113 |
| C-reactive protein (mg/dl) | 10.9 (6.81; 18.2) | 18.5 (11.4; 24.2) | 0.004 | 14.2 (9.08; 16.8) | 0.450 |
| Procalcitonin (ng/mL) | 0.85 (0.39; 2.87) | 4.54 (2.56; 7.63) | 0.003 | 0.48 (0.28; 3.33) | 0.640 |
| Albumin (g/L) | 3.50 (3.27; 4.00) | 3.40 (3.00; 3.80) | 0.153 | 3.65 (3.30; 3.92) | 0.779 |
| ALT (IU/L) | 50.0 (16.0; 103) | 24.0 (18.0; 44.0) | 0.061 | 34.0 (20.0; 69.0) | 0.662 |
| AST (IU/L) | 40.0 (27.0; 60.0) | 34.0 (26.0; 56.0) | 0.351 | 35.0 (23.0; 46.0) | 0.170 |
| GGT (IU/L) | 42.0 (20.8; 114) | 20.5 (15.0; 46.8) | 0.031 | 15.0 (13.5; 66.8) | 0.137 |
| Nt proBNP | 503 (356; 1,475) | 4,766 (3,046; 13,596) | < 0.001 | 776 (268; 1,260) | 0.849 |
| IL_6 | | 185 (75.4; 310) | | 82.4 (51.7; 84.6) | |
| D-dimer | 1,883 (671; 3,185) | 2,461 (1,041; 3,960) | 0.591 | 625 (287; 5,478) | 0.705 |
| Fibrinogen | 597 (534; 680) | 810 (518; 930) | 0.289 | 591 (523; 619) | 0.605 |
| Ferritin | 185 (128; 247) | 476 (358; 966) | < 0.001 | 153 (116; 588) | 0.479 |

[#]*This p-value compares 2018–2019 with 2020 (CoV–). *Not tested in 5 patients. CoV+, SARS-CoV-2 positive patients; CoV–, SARS-CoV-2 negative patients; ESR, Erythrocyte sedimentation rate; ALT, Alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; NT-proBNP, N-terminal pro b-type natriuretic peptide; IL-6, Interleukin 6.*

not fulfill AHA criteria when treatment was administered, finally fulfilled criteria for incomplete KD.

Patients CoV+ presented with significantly fewer lips and oral changes than PreCoV patients (85 vs. 50%, $p < 0.005$). No patient in the CoV+ group had sterile pyuria while it was observed in 21% of PreCoV and 35% of CoV– patients. Respiratory symptoms were not increased during COV-19p, and although it was more likely to have an abnormal chest X-ray in the CoV+ group, this was not significant. Overall, gastrointestinal symptoms were observed in 57% PreCoV vs. 65% in CoV+ and 55% CoV– with an increased proportion of patients with abdominal pain in the CoV+ group (33 vs. 58% and 30%). Irritability was significantly lower in the CoV+ group. Shock that was not related to IVIG infusion was observed in an increased number of patients during the CoV-19p (CoV– 4/20, 20%, and CoV+ 13/26,50%, $P < 0.001$) vs. 1.6% of patients during the PreCoV. Twenty-three (88.5%) of the CoV+ patients fulfilled both PIMS-TC and MIS-C criteria; from the CoV– cohort, 45% of patients fulfilled the criteria for MIS-C, and 40% for PIMS-TS, assuming all these patients would have had exposure to SARS-CoV-2 during this period, which is not recorded in the database (**Tables 4A**, **B**).

There were no meaningful differences in blood results between PreCoV and CoV– patients. Nevertheless, when

**TABLE 2 |** Detailed microbiological non SARS-CoV-2 results of patients with Kawasaki disease (2018–2019), 2020 (CoV+) and (CoV-).

| 2018–2019 | | 2020 | | | |
|---|---|---|---|---|---|
| | | | SARS-COV-2 POSITIVE | | |
| **Patient ($n = 63$)** | **Microbiology (Non SARS-CoV-2)** | **Patient ($n = 46$)** | **Microbiology (Non SARS-COV-2)** | **PCR SARS-COV-2** | **Serology SARS-COV-2** |
| 1 | (–) | 1 | (–) | (+) | Not tested |
| 2 | (–) | 2 | (–) | (–) | (+) |
| 3 | (–) | 3 | EBV & CMV[d] | (+) | Not tested |
| 4 | Enterovirus[b] | 4 | (–) | (+) | (+) |
| 5 | (–) | 5 | (–) | (+) | Not tested |
| 6 | (–) | 6 | (–) | (–) | (+) |
| 7 | Metapneumo & coronavirus[a] | 7 | (–) | (–) | (+) |
| 8 | (–) | 8 | (–) | (–) | (+) |
| 9 | (–) | 9 | (–) | (–) | (+) |
| 10 | (–) | 10 | (–) | (–) | (+) |
| 11 | (–) | 11 | (–) | (–) | (+) |
| 12 | *Salmonella*[c] | 12 | (–) | (+) | (+) |
| 13 | (–) | 13 | (–) | (+) | (+) |
| 14 | (–) | 14 | (–) | (–) | (+) |
| 15 | (–) | 15 | (–) | (–) | (+) |
| 16 | (–) | 16 | (–) | (–) | (+) |
| 17 | (–) | 17 | (–) | (–) | (+) |
| 18 | (–) | 18 | (–) | (–) | (+) |
| 19 | (–) | 19 | CMV & EBV in blood (+) | (+) | Not tested |
| 20 | (–) | 20 | (–) | (–) | (+) |
| 21 | (–) | 21 | Anti-HBc, & anti-HBs (+) | (+) | Not tested |
| 22 | (–) | 22 | (–) | (–) | (+) |
| 23 | (–) | 23 | (–) | (–) | (+) |
| 24 | (–) | 24 | (–) | (+) | (+) |
| 25 | (–) | 25 | (–) | (+) | (+) |
| 26 | (–) | 26 | (–) | (+) | (+) |
| 27 | (–) | | SARS-COV-2 NEGATIVE | | |
| 28 | (–) | 27 | (–) | (–) | (–) |
| 29 | (–) | 28 | *Mycoplasma*[d] | (–) | (–) |
| 30 | (–) | 30 | (–) | (–) | (–) |
| 31 | (–) | 31 | (–) | (–) | (–) |
| 32 | (–) | 32 | (–) | (–) | (–) |
| 33 | Rhinovirus[a] | 33 | (–) | (–) | (–) |
| 34 | (–) | 34 | Rhinovirus & enterovirus[a,b] | (–) | (–) |
| 35 | Parvovirus[d] | 35 | (–) | (–) | (–) |
| 36 | GAS[b] | 36 | (–) | (–) | (–) |
| 37 | Rhinovirus[a] | 37 | (–) | (–) | (–) |
| 38 | Rhinovirus[a] | 38 | Adenovirus[a] & *E.coli*[e] | (–) | (–) |
| 39 | Rhinovirus[a] | 39 | (–) | (–) | (–) |
| 40 | (–) | 40 | Bocavirus[a] | (–) | (–) |
| 41 | (–) | 41 | (–) | (–) | (–) |
| 42 | (–) | 42 | (–) | (–) | (–) |
| 43 | (–) | 43 | (–) | (–) | (–) |
| 44 | (–) | 44 | (–) | (–) | (–) |

*(Continued)*

**TABLE 2** | Continued

| 2018–2019 | | 2020 | | | |
|---|---|---|---|---|---|
| **Patient ($n = 63$)** | **Microbiology (Non SARS-CoV-2)** | **Patient ($n = 46$)** | **Microbiology (Non SARS-COV-2)** | **PCR SARS-COV-2** | **Serology SARS-COV-2** |
| 45 | (–) | 45 | (–) | (–) | (–) |
| 46 | GAS[b] | 46 | (–) | (–) | (–) |
| 47 | *Streptococcus* spp.[b] | 47 | (–) | (–) | (–) |
| 48 | (–) | | | | |
| 49 | (–) | | | | |
| 50 | GAS[b] | | | | |
| 51 | (–) | | | | |
| 52 | Parvovirus[d] | | | | |
| 53 | (–) | | | | |
| 54 | *Mycoplasma*[d] | | | | |
| 55 | (–) | | | | |
| 56 | (–) | | | | |
| 57 | (–) | | | | |
| 58 | (–) | | | | |
| 59 | (–) | | | | |
| 60 | (–) | | | | |
| 61 | CMV[d] | | | | |
| 62 | Adenovirus[a] | | | | |
| 63 | CMV[d] | | | | |

[a] *Nasopharyngeal aspirate.*
[b] *Pharyngeal swab.*
[c] *Stool culture.*
[d] *Serology (IgM).*
[e] *Urine culture.*
*(–), Negative; (+), Positive; GAS, Group A Streptococcus; CMV, Cytomegalovirus; EBV, Epstein Barr Virus; Anti-HBc, hepatitis B core antibody; Anti-HBs, hepatitis B surface antibody. All patients from the CoV– group were tested for SARS-COV-2 PCR and serology.*

comparing PreCoV to CoV+ patients we found in CoV+ group significantly lower leucocyte ($15.5 \times 10^9$/L vs. $9.5 \times 10^9$/L, $p < 0.001$) and platelet count ($423 \times 10^9$/L vs. $174 \times 10^9$/L, $p < 0.001$) and higher terminal segment of the natriuretic atrial peptide (NT-proBNP) (503 pg/ml vs. 4,766 pg/ml, $p < 0.001$), ferritin (185 ng/ml and 476 ng/ml, $p < 0.001$), C-reactive protein (CRP) (median, 10.9 vs. 18.5 mg/dl, $p < 0.005$), and procalcitonin (PCT) (median, 0.85 vs. 4.54 ng/ml, $p < 0.005$) (**Table 1**, **Figures 2**, **3**).

A positive microbiological finding other than SARS-CoV-2 was found in PreCoV, CoV+, and CoV– patients in 27, 15, and 20% of cases, respectively (**Table 2**).

## Outcome and Treatment

Echocardiographic examinations were abnormal in PreCoV, CoV–, and CoV+ patients in 37% (23/67), 45% (9/20), and 50% (13/26), respectively: CAL were observed in 18/23 (75%), 3/9 (33.3%), and 4/13 (39%) among those with abnormal echocardiography, coronary aneurysms in 7/63 (11%), 2/20 (10%), and 1/26 (3.8%), left ventricular dysfunction in 1/63 (1.59%), 2/20 (10%), and 8/26 (30.8%), and pericardial effusion in 13/23 (56.5%), 4/9 (44.4%), and 3/13 (23.1%). Left ventricular dysfunction was the only echocardiographic finding that was significantly higher in CoV+ than PreCoV group ($p < 0.001$) and in all cases it was transient. No giant coronary artery aneurysms were seen. A higher rate of abnormal repolarization in electrocardiographic studies was observed in CoV+ patients compared with PreCoV and CoV– patients (19 vs. 3% and 0%, respectively).

Treatment with IVIG was given to 95, 100, and 80% of the PreCoV, CoV–, and CoV+ patients, respectively. The mean days of fever onset to IVIG administration were 6 days in all groups. A second IVIG dose was given in 14% (8/58), 20% (4/20), and 19% (4/21), and corticosteroids were given in 28.6% (18/63), 50% (10/20), and 61.5% (16/26), respectively. Only one CoV+ and one CoV– patient received Tocilizumab, and one patient in the CoV+ group received Anakinra.

A significantly higher number of patients were admitted to PICU from the CoV+ 50% (13/26) vs. the PreCoV group 5% (3/63), $p < 0.001$. When comparing the PreCoV to the CoV– patients, there is still a higher non-significant number of patients that require PICU 5% (3/63) vs. 30% (6/20). The main reason for PICU admission in the CoV+ group was due to cardiac dysfunction or shock. Vasoactive support was given to 3/13 (23%) in the CoV+ group vs. 1/6 (16%) in the CoV– group and none during the PreCoVp.

Only one patient from PreCoV had a persistent aneurysm, and one in the CoV– is still under follow-up. No deaths were reported (**Table 3**).

**TABLE 3 |** Demographics and Clinical Features of patients with Kawasaki disease (2018–2019) vs. 2020 (*CoV+) and (CoV–*).

| | **2018–2019 N = 63** | **2020 (*CoV+*) n = 26** | ***p*. value** | **2020 *(CoV-)* N = 20** | ***p*. value** |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Male | 38 (62.3%) | 18 (69.2%) | 0.709 | 10 (50.0%) | 0.478 |
| Median age (years) (IQR) | 2.55 (1.46; 3.89) | 7.54 (5.36; 10.8) | <0.001 | 3.56 (2.20; 6.37) | 0.074 |
| Non-Caucasian | 18 (28.6%) | 16 (64.0%) | 0.005 | 5 (25.0%) | 0.981 |
| **Clinical features** | | | | | |
| Median days of fever | 7.00 (6.00; 8.00) | 7.00 (6.00; 9.00) | 0.483 | 7.00 (5.75; 8.00) | 0.817 |
| Complete KD | 43 (68.3%) | 7 (26.9%) | <0.001 | 8 (40.0%) | 0.019 |
| ***Classical criteria*** | | | | | |
| Conjunctival injection | 49 (77.8%) | 22 (84.6%) | 0.660 | 16 (80.0%) | 1.000 |
| Lips and oral changes | 53 (85.5%) | 13 (50.0%) | 0.001 | 16 (80.0%) | 0.725 |
| Changes in extremities | 41 (66.1%) | 8 (32.0%) | 0.008 | 7 (35.0%) | 0.028 |
| Polymorphous exanthema | 54 (87.1%) | 21 (80.8%) | 0.515 | 14 (70.0%) | 0.094 |
| Acute lymphadenopathy | 32 (50.8%) | 11 (42.3%) | 0.620 | 7 (35.0%) | 0.329 |
| ***Other symptoms*** | | | | | |
| **Respiratory symptoms** | 40 (63.5%) | 19 (73.1%) | 0.533 | 13 (65.0%) | 1.000 |
| Rhinorrhea | 21 (33.3%) | 0 (0.00%) | 0.002 | 2 (10.0%) | 0.081 |
| Cough | 15 (23.8%) | 4 (15.4%) | 0.550 | 3 (15.0%) | 0.540 |
| Wheezing | 2 (3.17%) | 1 (3.85%) | 1.000 | 1 (5.00%) | 0.568 |
| Pleural effusion | 2 (3.17%) | 1 (3.85%) | 1.000 | 0 (0.00%) | 1.000 |
| Dyspnea | 2 (3.17%) | 1 (3.85%) | 1.000 | 0 (0.00%) | 1.000 |
| Abnormal Chest-X-ray | 6 (17.6%) | 10 (38.5%) | 0.131 | 4 (25.0%) | 0.707 |
| **Musculoskeletal symptoms** | | | | | |
| Arthralgia | 8 (12.7%) | 1 (3.85%) | 0.274 | 1 (5.00%) | 0.680 |
| Arthritis | 8 (12.7%) | 1 (3.85%) | 0.274 | 1 (5.00%) | 0.680 |
| Myalgia | 4 (6.35%) | 3 (11.5%) | 0.412 | 0 (0.00%) | 0.568 |
| Gastrointestinal symptoms | 36 (57.1%) | 17 (65.4%) | 0.629 | 11 (55.0%) | 1.000 |
| Abdominal pain | 21 (33.3%) | 15 (57.7%) | 0.059 | 6 (30.0%) | 0.997 |
| Nausea | 4 (6.35%) | 3 (11.5%) | 0.412 | 2 (10.0%) | 0.628 |
| Vomits | 23 (36.5%) | 10 (38.5%) | 1.000 | 5 (25.0%) | 0.498 |
| Diarrhea | 8 (12.7%) | 6 (23.1%) | 0.336 | 4 (20.0%) | 0.471 |
| Any hepatic alteration | 30 (47.6%) | 14 (53.8%) | 0.763 | 9 (45.0%) | 1.000 |
| Hypertransaminasemia | 30 (47.6%) | 13 (50.0%) | 1.000 | 8 (40.0%) | 0.735 |
| Hyperbilirubinemia | 4 (6.35%) | 1 (3.85%) | 1.000 | 0 (0.00%) | 0.568 |
| Hepatomegaly | 4 (6.35%) | 2 (7.69%) | 1.000 | 0 (0.00%) | 0.568 |
| Cholestasis | 1 (1.59%) | 1 (3.85%) | 0.501 | 0 (0.00%) | 1.000 |
| **Nervous system symptoms** | | | | | |
| Headache | 6 (9.68%) | 3 (11.5%) | 0.688 | 1 (5.26%) | 1.000 |
| Irritability | 35 (55.6%) | 1 (3.85%) | <0.001 | 9 (47.4%) | 0.271 |
| Aseptic meningitis | 2 (3.17%) | 1 (3.85%) | 0.328 | 0 (0.00%) | 0.707 |
| **Genitourinary symptoms** | | | | | |
| Hematuria | 4 (6.35%) | 2 (7.69%) | 0.680 | 3 (15.0%) | 0.218 |
| Sterile pyuria | 13 (21.3%) | 0 (0.00%) | 0.015 | 7 (35.0%) | 0.427 |
| **Shock not related with IVIG** | 1 (1.59%) | 13 (50.0%) | <0.001 | 4 (20.0%) | 0.011 |
| **PIMS-TS criteria fulfilled** | – | 23 (88.5%) | | 8 (40%) | |
| **Complete KD** | | 7/23 (30.4%) | | 3/8 (37.5%) | |
| **Incomplete KD** | | 16/23 (69.6%) | | 5/8 (62.5%) | |
| **MIS-C criteria fulfilled** | – | 23 (88.5%) | | 9 (45%) | |
| **Complete KD** | | 7/23 (%) | | 2/9 (22.2%) | |
| **Incomplete KD** | | 16/23 (%) | | 7/9 (77.8%) | |
| **Cardiology examinations** | | | | | |
| Any echocardiogram alterations | 23 (37.1%) | 13 (50.0%) | 0.376 | 9 (45%) | 0.594 |

*(Continued)*

**TABLE 3 |** Continued

| | 2018–2019 *N* = 63 | 2020 (*CoV+*) *n* = 26 | *p*. value | 2020 *(CoV-)* *N* = *20* | *p*. value |
|---|---|---|---|---|---|
| Coronary artery lesions | 18 (75.0%) | 4 (30.8%) | 0.023 | 3 (33.3%) | 0.044 |
| Ectasia | 12 (19.0%) | 3 (11.5%) | 0.538 | 2 (10.0%) | 0.500 |
| Aneurysm | 7/23 (30.4%) | 1/13 (7.7%)* | 0.212 | 2/9 (22.2%) | 1.000 |
| | 7/63 (11.1%) | 1/26 (3.85%) | 0.429 | 2/20 (10.0%) | 1.000 |
| z score: (small) | 7 (100%) | 1 (100%) | | 2 (100%) | . |
| Persistent Aneurysm | 1/7 (14.3%) | 0 (0.00%) | 0.250 | 0 (0.00%) | 0.417 |
| Systolic dysfunction Left V. | 1/63 (1.59%) | 8/26 (30.8%) | <0.001 | 2/20 (10.0%) | 0.143 |
| Pericardial effusion | 13/23 (56.5%) | 3/13 (23.1%) | 0.083 | 4/9 (44.4%) | 0.699 |
| Repolarization alterations | 2 (3.17%) | 5 (19.2%) | 0.021 | 0 (0.00%) | 1.000 |
| **Pharmacotherapy** | | | | | |
| Days from fever to IVIG | 6.00 (5.00; 8.00) | 6.00 (4.00; 7.00) | 0.194 | 6.00 (5.00; 8.25) | 0.455 |
| IVIG 1st dose | 58/63 (92%) | 21/26 (80.8%) | 0.149 | 20 (100%) | 0.329 |
| IVIG 2nd dose | 8/58 (13.8%) | 4/21 (19.0%) | 0.723 | 4 (20.0%) | 0.492 |
| Corticosteroids | 18/63 (28.6%) | 16/26 (61.5%) | 0.008 | 10 (50.0%) | 0.104 |
| Tocilizumab | 0 (0.00%) | 1 (33.3%) | 1.000 | 1 (100%) | 1.000 |
| Anakinra | 0 (0.00%) | 1 (14.3%) | 1.000 | 0 | – |
| **Outcome** | | | | | |
| Admission to PICU | 3 (4.92%) | 13 (50.0%) | <0.001 | 6 (30.0%) | 0.006 |
| Days in PICU | 7.00 (5.50; 7.50) | 4.00 (3.00; 5.00) | 0.247 | 4.50 (2.25; 6.75) | 0.362 |
| **Reason PICU admission** | | | | | |
| Myocarditis | 0 (0.00%) | 6 (23.1%) | <0.001 | 1 (5.00%) | 0.241 |
| Cardiac dysfunction | 0 (0.00%) | 6 (23.1%) | <0.001 | 0 | – |
| Suspicion of Sepsis | 2 (3.17%) | 1 (3.85%) | 1.000 | 0 (0.00%) | 1.000 |
| Hypotension shock | 1 (1.59%) | 9 (34.6%) | <0.001 | 3 (15.0%) | 0.042 |
| Vasoactive drugs: | 0 (0.00%) | 3 (11.5%) | 0.023 | 1 (5.00%) | 0.241 |
| Cardiogenic shock | 0 (0.00%) | 1 (3.85%) | 0.292 | 0 | - |
| Respiratory distress | 1 (1.59%) | 0 (0.00%) | 1.000 | 0 (0.00%) | 1.000 |

*Median (interquartilic range). CoV+, SARS-CoV-2 positive patients; CoV–, SARS-CoV-2 negative patients; IQR, interquartile range; KD, Kawasaki Disease; IVIG, intravenous immunoglobulin; Left V, Left Ventricle; MIS-C, multisystem inflammatory syndrome in children; PICU, pediatric intensive care unit; PIMS-TS, pediatric inflammatory multisystemic syndrome temporary associated with SARS-CoV-2.*

**This patient received IVIG within the first 10 days after fever onset.*

## DISCUSSION

To our knowledge, this is the first study to compare KD cases prospectively recruited presenting before and during the pandemic and that study separately the SARS-CoV-2 confirmed positive and negative cases and compare them with pre-pandemic patients from the preceding years.

We report a significant increase in the number of KD cases in Spain during the SARS-CoV-2 pandemic, with an overall 2-fold increase in cases reported as KD in the national database and a 10–20-fold increase of cases admitted to PICU compared to the previous 2 years. A similar study from northern Italy found a 30-fold increased incidence (13). Despite a 2-fold increase in the cases reported as KD in the national database when we analyzed each case in this new context, we find that classical KD remains similar, as the prevalence of CoV–KD seems not to have increased. Moreover, our series provides data strongly suggesting that even in the era of COVID-19 some classical KD remain and must not be considered as PIMS.

The majority of the KD SARS-CoV-2 confirmed cases fulfilled the PIMS-TC/MIS-C criteria; knowing case definition was intended to be sensitive, and therefore most KD cases are included. Albeit a positive result, detecting SARS-CoV-2 is suggestive for PIMS-TS/MIS-C, but it is not essential for diagnosis: a likely contact with patients with COVID-19 is enough for PIMS-TS, and MIS-C criteria include negative PCR for SARS-CoV-2.

Clinical and laboratory features of CoV+ KD patients resemble PIMS-TC/MIS-C and have many significant differences from PreCoV cases. Patients with CoV+ KD are older, have non-Caucasian predominance, more intense inflammation, and greater myocardial injury than patients with classical KD.

We have compared our data to other European KD series that also looked at KD and Kawasaki-like cases presenting during the

**TABLE 4A |** Patients from CoV+ cohort, indicating complete/incomplete KD and the criteria met for PIMS-TS and MIS-C diagnosis.

| **Patient number** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Complete/Incomplete KD | C | C | I | I | I | C | C | * | * | C | I | C | I | I | * | I | I | I | * | I | I | I | C | I | I | * |
| Fever > 3 d | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Rash/Conjunctivitis/Mucocutaneous inflammation signs | x | x | x | x | x | x | x | x | x | x | | x | x | x | x | x | x | x | x | x | | x | x | x | x | |
| Hypotension/Shock | x | x | x | | | x | x | | | | | | | x | x | x | | | | x | x | x | x | | | x |
| Myocardial dysfunction/pericarditis/valvulitis/CAA | x | x | x | | | x | x | x | x | x | x | x | | x | x | x | | x | x | x | x | x | | x | x | x |
| Coagulopathy | | x | x | x | | x | x | | | x | | | | x | x | x | x | | x | | x | x | x | | | |
| GI symptoms | | x | x | x | # | x | x | # | | | | ‡ | | x | x | x | x | x | x | x | x | x | | x | x | x |
| PCR > 5 mg/dL | | x | x | x | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| PCT > 1 ng/mL | x | x | x | x | | | x | x | x | x | | x | x | x | x | x | | x | x | x | x | | | | x | x |
| No other cause | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **PIMS-TS (WHO)** | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes | No | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Persistent fever | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Persistent inflammation | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Single or multi-organ dysfunction | x | x | x | x | # | x | x | #‡ | x | x | x | x | | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Shock | x | x | x | | | x | x | | | | | | | x | x | x | | | | x | x | x | x | | | x |
| Cardiac disorder | | x | x | | | x | x | | x | | x | | | x | x | x | | x | x | x | x | x | | x | x | |
| Respiratory disorder | | | | | | | | | | | | | | | | | | | x | x | | | | | | x |
| Renal disorder | | | | | | | | | | | | | | | | | | | | | | | | | | |
| GI disorder | | x | x | x | # | x | x | #‡ | | | | ‡ | | x | | x | x | x | x | x | x | x | | x | x | x |
| Neurological disorder | | | | | | | | | | x | x | x | | | | | | | | | | | | | | |
| No other cause | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| SARS-CoV-2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **MIS-C (RCPCH)** | Yes | Yes | Yes | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

*BP, Blood pressure; C, Complete; CAA, Coronary artery abnormalities; GI, Gastrointestinal; I, Incomplete; KD, Kawasaki disease; MIS-C, Multisystem inflammatory syndrome in children; PIMS-TS, Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2; RCPCH, Royal College of Paediatrics and Child Health; WHO, World Health Organization.*

**These patients didn't meet the criteria for KD at the moment of admission, but they fulfilled the criteria for incomplete KD during admission.*

*# These patients complained only of mild abdominal pain.*

*‡ These patients had elevation of liver enzymes but <2 upper limit of normality.*

**TABLE 4B |** Patients from CoV- cohort indicating complete/incomplete KD and the criteria met for PIMS-TS and MIS-C diagnosis.

| Patient number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Complete/Incomplete KD | I | I | C | C | I | I | C | * | I | I | I | I | C | I | C | I | C | I | C | C |
| Fever > 3 d | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Rash/ Conjunctivitis/Mucocutaneous inflammation signs | x | x | x | x | x | x | x | x | x | x | | x | x | x | x | x | x | x | x | x |
| Hypotension/Shock | | x | | | | | | x | x | | | | | | | | | x | | |
| Myocardial dysfunction/pericarditis/valvulitis/ CAA | x | x | | x | x | | | x | | | x | | x | | x | x | x | x | | |
| Coagulopathy | | x | | | | | | | | | | | | x | x | | | x | | |
| GI symptoms | x | x | | | x | x | | x | | | | | x | | x | x | x | x | x | |
| PCR> 5 mg/dL | x | x | | x | | x | | x | x | x | x | x | x | x | x | x | x | x | x | x |
| PCT > 1 ng/mL | | | | | | | | x | x | | | | | | x | | | x | | x |
| No other cause | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **MIS-C (WHO)** | Yes | Yes | No | No | No | No | No | Yes | No | No | No | No | Yes | No | Yes | Yes | Yes | Yes | No | No |
| Persistent fever | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Persistent inflammation | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Single or multi-organ dysfunction | x | x | | | x | | | x | x | | x | | x | | x | x | | x | | ‡ |
| Shock | | x | | | | | | x | x | | | | | | | | | x | | |
| Cardiac disorder | x | x | | | | | | x | | | x | | x | | x | x | | x | | |
| Respiratory disorder | | | | | | | | | | | | | x | | x | | | | | |
| Renal disorder | | | | | | | | | | | | | | | | | | | | |
| GI disorder | x | x | | | x | # | | # | | | | | x | | x | x | | x | | ‡ |
| Neurological disorder | | | | | | | | | | | | | | | | | | | | |
| No other cause | x | x | x | x | x | x | | x | x | x | x | x | x | x | x | x | x | x | x | x |
| SARS-CoV-2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **PIMS-TS (RCPCH)** | Yes | Yes | No | No | Yes | No | No | No | Yes | No | Yes | No | Yes | No | Yes | Yes | No | Yes | No | No |

*BP, Blood pressure; C, Complete; CAA, Coronary artery abnormalities; GI, Gastrointestinal; I, Incomplete; KD, Kawasaki disease; MIS-C, Multisystem inflammatory syndrome in children; PIMS-TS, Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2; RCPCH, Royal College of Paediatrics and Child Health; WHO, World Health Organization.*

**These patients didn't meet the criteria for KD at the moment of admission, but they fulfilled the criteria for incomplete KD during admission.*

*#These patients complained only of abdominal pain.*

*‡These patients had elevation of liver enzymes but <2 upper limit of normality.*

***Diagnostic criteria of inflammatory:***

- *MIS-C (WHO):*
  - *Children and adolescents 0–19 years of age with fever > 3 days AND two of the following:*

*a) Rash or bilateral non-purulent conjunctivitis or mucocutaneous inflammation signs (oral, hands, or feet).*

*b) Hypotension or shock.*

*c) Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including ECHO findings or elevated troponin/NT-proBNP)*

*d) Evidence of coagulopathy (by PT, PTT, elevated D-Dimers).*

*e) Acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain).*

  - *AND elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin.*
  - *AND no other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock síndrome.*
  - *AND evidence of COVID-19 (RT-PCR, antigen test, or serology positive), or likely contact with patients with COVID-19.*
- *PIMS-TS (RCPCH):*
  - *A child presenting with persistent fever, inflammation (neutrophilia, elevated CRP and lymphopaenia) and evidence of single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) with other additional clinical, laboratory or imagining and ECG features. Children fulfilling full or partial criteria for Kawasaki disease may be included.*
  - *Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus.*
  - *SARS-CoV-2 PCR testing positive or negative.*

pandemic. **Table 5** summarizes data from Pouletty et al. (15), Verdoni et al. (13), and Toubiana et al. (23).

The median PreCoV age was 2.55 years that was similar to the CoV– group (3.56 yr) and to our retrospective data (24) and significantly higher for CoV+ patients (7.54 yr) that was closer in age to those reported in the PIMS-TC series (9 yr) (5, 25) and the KD series during the pandemic (7.5–10 yr) (13, 15, 26–28).

Historically, KD has known to have male predominance (18), and we found a male predominance in PreCoV and in CoV+

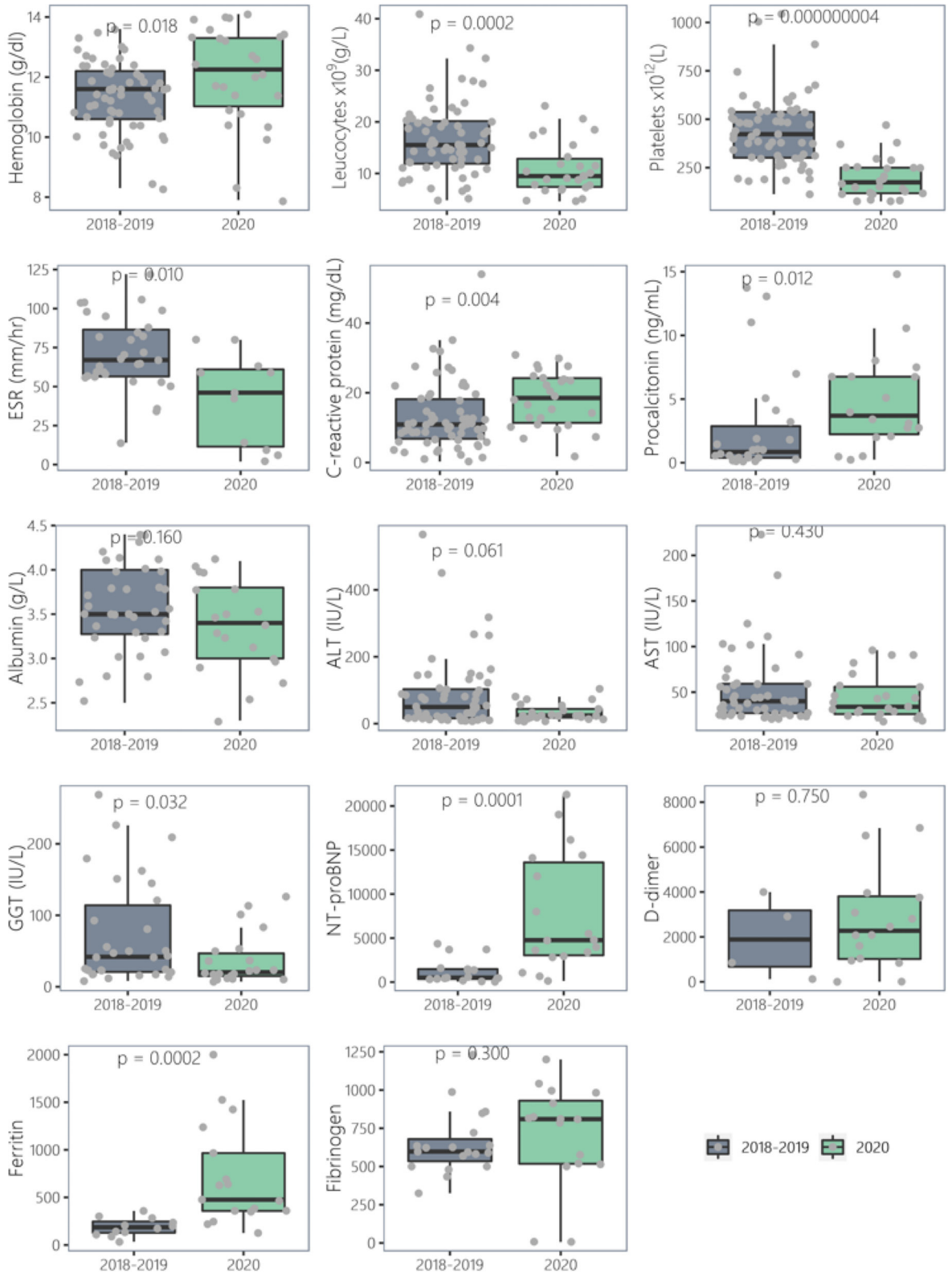


**FIGURE 2 |** Comparison of laboratory results of Kawasaki disease cases (2018–2019) vs. 2020 (CoV+).

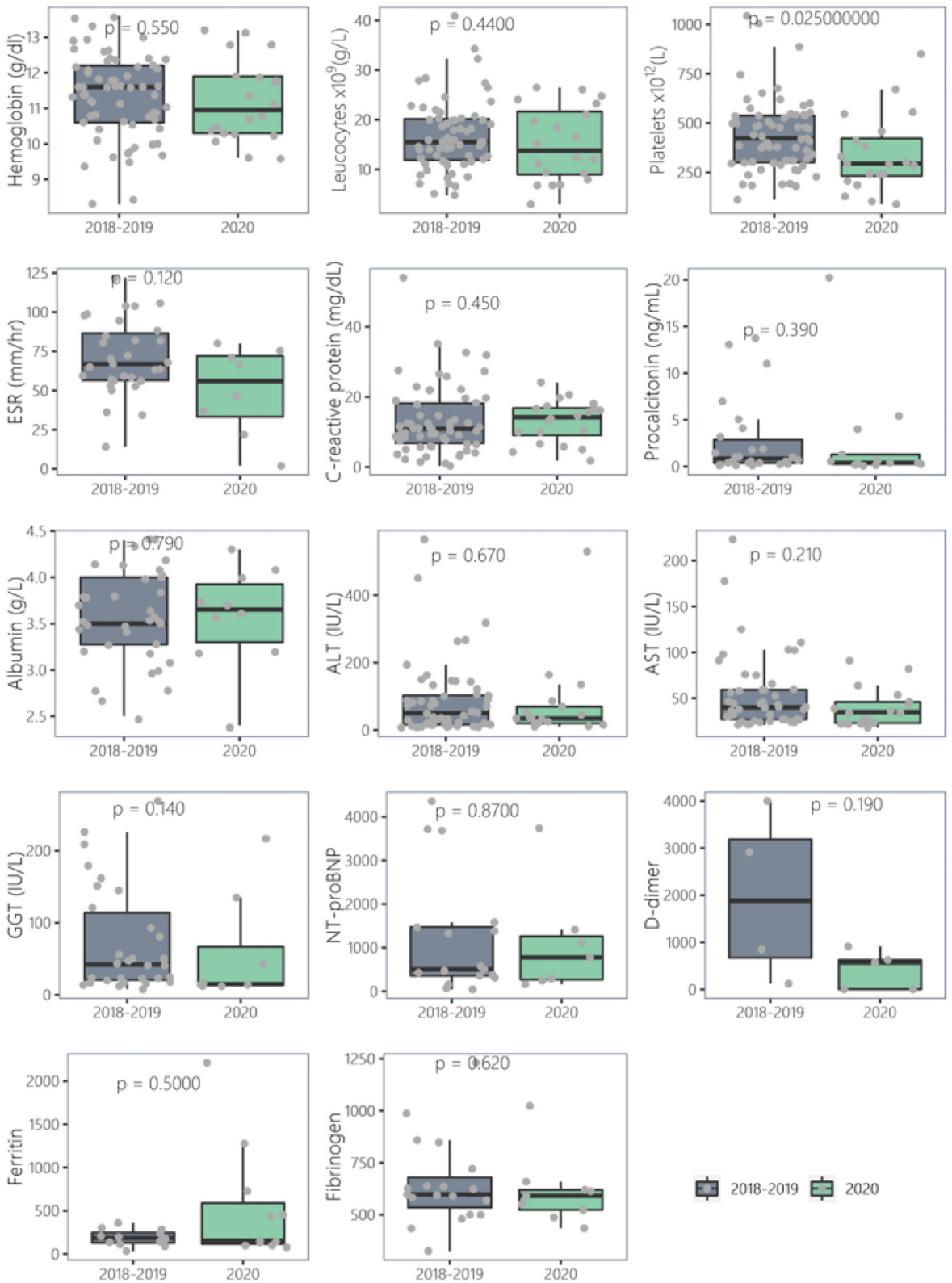


**FIGURE 3 |** Comparison of laboratory results of Kawasaki disease cases (2018–2019) vs. 2020 (CoV–).

**TABLE 5 |** Summary from Kawasaki disease series during the SARS-CoV-2 pandemic.

| | **Fernández-Cooke et al.* Our Cohort COVID + *N* = 26** | **Fernández-Cooke et al.* Our Cohort COVID – *N* = 20** | **Pouletty et al. (15)* *N* = 16** | **Verdoni et al. (13)** *N* = 10** | **Toubiana et al. (23)*** *N* = 21** |
|---|---|---|---|---|---|
| Median age (years) | 7.54 (5.36–10.8) | 3.56 (2.2–6.37) | 10 (4.7–12.5) | 7.5 (3.5) | 7.9 (3.7–16.6) |
| Gender-male *n* (%) | 18 (69.2%) | 10 (50%) | 8 (50%) | 7 (70%) | 9 (43%) |
| **Ethnicity** | | | | | |
| - Caucasian | 9 (36%) | 15 (75%) | 4 (25%)‡ | – | 12 (29%)‡‡ (*n* = 42) |
| - Not Caucasian | 16 (64%) | 5 (25%) | – | – | – |
| **Symptoms** | | | | | |
| Fever | 26 (100%) | 20 (100%) | 16 (100%) | 10 (100%) | 21 (100%) |
| Polymorphous exanthema | 21 (80.8%) | 14 (70%) | 13 (81%) | 8 (80%) | 16 (76%) |
| Conjunctival injection | 22 (84.6%) | 16 (80%) | 15 (94%) | 9 (90%) | 17 (81%) |
| Lips and oral changes | 13 (50%) | 16 (80%) | 14 (87%) | 7 (70%) | 16 (76%) |
| Changes in extremities | 8 (32%) | 7 (35%) | 11 (68%) | 5 (50%) | 10 (48%) |
| Cervical lymphadenopathy | 11 (42.3%) | 7 (35%) | 6 (37%) | 1 (10%) | 12 (57%) |
| Complete KD | 7 (26.9%) | 8 (40%) | 10 (62%) | 5 (50%) | 11 (52%) |
| Gastrointestinal symptoms | 17 (65.4%) | 11 (55.0%) | 13 (81%) | 6 (60%) | 21 (100%) |
| Respiratory symptoms | 19 (73.1%) | 13 (65.0%) | 2 (12%) | – | – |
| Neurological symptoms | – | – | 9 (56%) | – | 12 (57%) |
| Musculoskeletal symptoms | – | – | 1 (6%) | 4 (40%) | 2 (10%) |
| **Imaging** | | | | | |
| Abnormal chest X-ray | 10 (38.5%) | 4 (25%) | 5 (31%) | 5 (50%) | 8 (44%) (*n* = 18) |
| **Blood tests** | | | | | |
| Hemoglobin (g/dL) | 12.2 (11–13.3) | 10.9 (10.3–11.9) | – | 11 (1.2) | 8.6 (5.3–12.2) |
| Leucocytes ($\times 10^9$/L) | 9.5 (7.4–12.8) | 13.8 (8.98–21.7) | 11.5 (9–14.4) | 10.8 (6.1) | 17.4 (5.4–42.8) |
| Platelets ($\times 10^9$/L)‡‡‡ | 174 (118–250) | 290 (193–423) | 188 (164–244) | 130 (32) | 499 (78–838) |
| C-Reactive Protein (mg/dL) | 18.5 (11.4–24.2) | 14.2 (9.08–16.8) | 20.7 (16.2–23.6) | 25 (15.3) | 25.3 (8.9–36.3) |
| Procalcitonin (ng/mL) | 4.54 (2.56–7.63) | 0.48 (0.28–3.33) | – | – | 22.5 (0.1–448) |
| ESR (mm/h) | 46 (11.5–61) | 56 (33.2–72) | – | 72 (24) (n=8) | – |
| Albumin (g/dL) | 3.4 (3–3.8) | 3.65 (3.3–3.92) | 2.1 (1.9–2.3) | 3.2 (0.3) | 2.1 (1.6–3.7) |
| ALT (IU/L) | 24 (18–44) | 34 (2–69) | – | 119 (217) | 70 (6–257) |
| AST (IU/L) | 34 (26–56) | 35 (23–46) | – | 87 (70) | – |
| GGT (IU/L) | 20.5 (15–46.8) | 15 (13.5–66.8) | – | – | 59 (10–205) |
| NT-proBNP (ng/L) | 4,766 (3,046–13,596) | 776 (268–1,260) | BNP ng/L (4,319, 2,747–6,493) | 1,255 (929) | BNP ng/L (3,354, 16–16,017) (*n* = 18) |
| D-dimer (mcg/L) | 2,461 (1,041–3,960) | 625 (287–5,478) | – | 3,798 (1,318) | 4,025 (350–19,330) (*n* = 20) |
| Interleukin 6 (pg/mL) | 185 (75.4–310) | 82.4 (51.7–84.6) | 270 (136–526) | 177.1 (137.4) (*n* = 4) | 170 (4–1,366) (*n* = 17) |
| Fibrinogen (mg/dL) | 810 (518–930) | 591 (523–619) | – | 621 (182) | – |
| Ferritin (ng/mL) | 476 (358–966) | 153 (116–588) | 1,067 (272–1,709) | 1,176 (1,032) | – |
| **Microbiological results** | | | | | |
| SARS-CoV-2 PCR + | 11 (42.3%) | 0 | 9 (56) | 2 (20%) | 8 (38%) |
| SARS-CoV-2 serology (IgG and/or IgM) + | 21 (100%) (*n* = 21) | 0 | 7/8 (87%) | 8 (80%) | 19 (90%) (*n* = 20) |
| **Pharmacotherapy** | | | | | |
| IVIG n (%) | 21 (80.8%) | 20 (100%) | 15 (93%) | 10 (100%) | 21 (100%) |
| Steroids | 16 (61.5%) | 10 (50%) | 4 (25%) | 8 (80%) | 10 (48%) |
| Resistance to IVIG (persistence of fever >36 h) | 4 (19%) (*n* = 21) | 4 (20%) | 10 (62%) | 10 (100%) | 5 (24%) |
| **Cardiology examinations** | | | | | |
| Abnormal echocardiography | 13 (50%) | 9 (47.4%) | 11 (69%) | 6 (60%) | – |
| Coronary artery dilations | 3 (11.5%) | 2 (10%) | 3 (19%) | – | 5 (24%) |
| Coronary artery aneurisms | 1 (3.85%) | 2 (10%) | 0 | 2 (20%) | 0 |
| Myocarditis | 6 (23.1%) | 1 (5%) | 7 (43.8%) | 5 (50%) § | 16 (76%) |

*(Continued)*

**TABLE 5** | Continued

| | Fernández-Cooke et al.* Our Cohort COVID + *N* = 26 | Fernández-Cooke et al. Our Cohort COVID – *N* = 20 | Pouletty et al. (15)* *N* = 16 | Verdoni et al. (13)** *N* = 10 | Toubiana et al. (23)*** *N* = 21 |
|---|---|---|---|---|---|
| Pericarditis / pericardial effusion | 3 (23.1%) | 4 (44.4%) | 4 (25%) | 4 (40%) | 10 (48%) |
| **Outcome** | | | | | |
| Admission to PICU | 13 (50%) | 6 (30%) | 7 (44%) | – | 17 (81%) |
| Shock | 13 (50%) | 4 (20%) | 7 (44%) | 5 (50%) | 12 (57%) |

*Median (interquartilic range).
**Results as mean (standard deviation).
***Results as median (range).
†From Europe, not indicated Caucasian.
‡‡Parents, not patients, and from Europe, not indicated Caucasian.
‡‡‡Corresponding in the different studies to:
- Cooke et al. COVID+ Platelets at admission are equal to minimum platelets.
- Cooke et al. COVID- Minimum platelets (at admission 294, 193–423).
- Pouletty et al. Minimum platelets.
- Verdoni et al. not indicated.
- Toubiana et al. not indicated.
§Indicated ejection fraction < 55%.
ALT, Alanine Aminotransferase; AST, Aspatate Aminotransferase; ESR, Erythrosedimentation Rate; GGT, Gamma Glutamyltransferase; IVIG, intravenous immunoglobulin; KD, Kawasaki Disease; NT-proBNP, N-Terminal proB-type Natriuretic Peptide; PICU, Pediatric Intensive Care Unit.

patients. African Americans have been affected by the COVID-19 pandemic at a disproportionately higher rate (29).

Interestingly, we found a significantly higher proportion of non-Caucasians in the CoV+ group, which does not represent the general Spanish population. Additionally, our retrospective study on the same population (2011–2016) found 76% of KD cases had European origin (24). Other studies have also found over-representation of non-Caucasian patients in the KD series during the pandemic (5, 15, 23) reaching in a published PIMS-TC cohort 100% of patients (25), and this may suggest an effect of either social and living conditions or genetic susceptibility (30).

The CoV+ group had more likely incomplete KD than in the PIMS-TS series (5, 23, 31). This phenomenon could be because PIMS-TC and CoV+ KD are a separate entity or because pediatricians are more aware now and diagnose more incomplete KD in this context. After conjunctival injection, the most frequent classical symptom was erythematous rash and significantly less common lips and oral changes in CoV+ patients; this was also observed in PIMS-TC patients (5, 23, 31). No patient in the CoV+ group had sterile pyuria that is typically observed in classical KD patients.

Respiratory symptoms were not increased in our series, and although not significant, it was more likely to have an abnormal chest X-ray during the pandemic as reported by Toubiana et al. (23) (44%). Probably the small sample size in our series does not allow us to detect significant differences. There was an increased proportion of patients with abdominal pain in the CoV+ group, and this symptom was widely observed in PIMS-TC patients (5, 25). Irritability was significantly lower in the CoV+ group, probably due to the higher median age in this group. Shock that was not related to IVIG infusion was observed in a significantly increased number of patients who were CoV+. This phenomenon was also observed by the other KD pandemic series (**Table 5**), probably indicating again that these patients are on the PIMS-TC spectrum.

Surprisingly, there is an absence of reported cases of Kawasaki-like MIS associated with SARS-CoV-2 infection in Asian countries where the COVID-19 pandemic started, and where the incidence of KD was the highest. It has been hypothesized that a mutation from a European strain of SARS-CoV-2 drives a stronger cytokine storm. Serology was more likely to be positive than PCR in our series, supporting the postinfectious hypothesis, because the disease appears to occur 2–4 weeks after acute SARS-CoV-2 infection or exposure. This has also been observed in the patients with PIMS-TC and those with heart failure in this context (23, 25–27). The coronavirus family might represent one of the triggers of KD as previously hypothesized (32, 33), and the SARS-CoV-2 spike may act as a superantigen driving a cytokine storm that leads to hyperinflammation (14, 34). A positive microbiological finding other than SARS-CoV-2 was found in around 20–25% of the PreCoV and CoV– patients. Some of the CoV– negative patients could have had a different trigger despite the lockdown, but some may have been CoV false negatives explaining why this group has overlapping clinical and laboratory features. There were no meaningful differences in blood results between PreCoV and CoV– patients. Nevertheless, when comparing PreCoV to CoV+ patients, we found significantly lower leucocyte and platelet count and higher NT-proBNP, ferritin, CRP, and PCT in CoV+ patients as found in other cohorts (13, 23). These findings are closer to those exhibited by the PIMS-TC/MIS-C and KDSS (35) patients than to classical KD patients. The NT-proBNP levels we found were more in line with the Paris Kawa-COVID-19 cohort (median 4,319 pg/mL) (15) in contrast with Belhadjer et al. that reported 10 times higher levels (mean 41,484 pg/mL) in PIMS-TC patients (26).

Echocardiographic examinations were abnormal in 50% of CoV+ patients similar to other KD pandemic series (**Table 5**) and higher than classical KD cases; this is probably because many CoV+ patients presented with left ventricular dysfunction.

CAL, defined as Z-score > 2, were observed in a third of our patients presenting during the pandemic, but <10% had coronary aneurysms, and this was less likely in the CoV+ group. In PIMS-TC patients, 7–12% present aneurysms (25). Interestingly, there are at least three studies that report no coronary aneurysms in Kawa-COVID-19 patients (15) and PIMS-TC patients (23, 26); in absolute number, we only had one patient in the CoV+ group with a coronary aneurysm. Our impression is that the cases associated with SARS-CoV-2, and although they have more cardiac involvement, it is more likely to be left ventricular dysfunction than cardiac aneurysms, but this needs to be studied further. It is known that older children with KD and intense inflammation are more likely to present with myocarditis (36). Children with PIMS-TC have been described to have mild to moderate heart dysfunction indicating acute myocardial injury and recovering normal cardiac function within a week, probably because there is inflammation and myocardial oedema but without myocardial necrosis (31, 37). Left ventricular dysfunction was the only echocardiographic finding that was significantly higher in CoV+, and all cases were transient. Abnormal repolarization was relatively frequent in CoV+ patients in our study; this is probably linked to the higher proportion of patients with left ventricular dysfunction. A range of ECG abnormalities (14–60%) have been reported in PIMS-TC patients (5, 25).

We found that while 100% of CoV– patients received IVIG, this drops to 80% in the CoV+ patients. We think this is because some may have been treated as PIMS-TC/MIS-C where some clinicians gave steroids directly or they recovered with supportive care alone. Although there are no studies yet on the best treatment for this new entity, most groups are giving first-line treatment with IVIG followed by steroids in some patients ranging from 33 to 64%, and biological agents in 8–14% (5, 23, 25, 26). A second IVIG dose was given in a similar proportion of patients from all groups, and corticosteroids were given more frequently in CoV+ patients, probably due to higher inflammation markers and suspicion of PIMS-TC.

A significantly higher number of patients were admitted to PICU from the CoV+ group. The reason for PICU admission was due to cardiac dysfunction or shock resembling again PIMS-TC rather than classical KD (5, 23, 25). The mean length of the PICU stay was generally under a week (4–5 days) (23, 25, 27). Only one patient from PreCoV had a persistent aneurysm, and one in the CoV- is still under follow-up. No deaths were reported.

Our study has some potential limitations. There is a potential recruitment bias that may have contributed to the high number of patients with Kawasaki-like multisystem inflammatory syndrome, as pediatricians have been more aware due to the alerts. Antibody tests against SARS-CoV-2 were performed by different techniques depending on the Hospital; therefore, sensitivity and specificity have a broad range, driving to a potential underdiagnosis. We could not calculate an overall incidence of KD because our network did not achieve total national coverage. Nevertheless, we think that the number of patients collected allows us to draw conclusions, and the prospective character of our network KAWA-RACE before and during the pandemic places us in a privileged setting to have an overview of what has happened in Spain.

In summary, we describe findings in the CoV+ group and remark clinical and laboratory differences to classify them as PIMS-TC/MIS-C and not classical KD (higher median age, non-Caucasian predisposition, more likely incomplete presentation, more myocardial dysfunction, less aneurysm development, more PICU admission, and higher inflammatory markers). Whether this is a novel entity or the same disease on different ends of the spectrum is yet to be elucidated. However, SARS-CoV-2 seems to be a potent trigger that in some patients leads to an aberrant immune response, especially in older children, and may be due to previous infections.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, under reasonable request.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Instituto de Investigacion Hospital 12 de Octubre approved this study (CEIC 15/316). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

EF-C, CG, ABa, JS-M, BM, EV, MC, MN, MO, GG, MB, ABe, BR, MR-G, EN, JAr, and DM contributed to the acquisition of data. EF-C, CG, SD-R, ABa, JS-M, JAn, and CC contributed to the analysis and interpretation of the data and drafted the manuscript. EF-C, CG, ABa, JS-M, JAn, and CC contributed to the conception and design of the manuscript. BM, EV, MC, MN, MO, GG, MB, ABe, BR, MR-G, EN, JAr, and DM critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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# Lower CMV and EBV Exposure in Children With Kawasaki Disease Suggests an Under-Challenged Immune System

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**Background:** Kawasaki Disease (KD) is a pediatric vasculitis of which the pathogenesis is unclear. The hypothesis is that genetically pre-disposed children develop KD when they encounter a pathogen which remains most often unidentified or pathogen derived factors. Since age is a dominant factor, prior immune status in children could influence their reactivity and hence the acquisition of KD. We hypothesized that systemic immune responses early in life could protect against developing KD. With this study we tested whether the incidence of previous systemic cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection is lower in children with KD compared to healthy age-matched controls.

**Methods and Results:** We compared 86 KD patients with an age-matched control group regarding CMV and EBV VCA IgG measurements (taken before or 9 months after IVIG treatment). We found that both CMV and EBV had an almost 2-fold lower seroprevalence in the KD population than in the control group.

**Conclusions:** We suggest that an under-challenged immune system causes an altered immune reactivity which may affect the response to a pathological trigger causing KD in susceptible children.

**Keywords: Kawasaki disease, Epstein-Barr virus, immune system, viral exposure, cytomegalovirus**

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## INTRODUCTION

Kawasaki disease (KD) is a rare pediatric vasculitis of unknown etiology that can lead to coronary artery aneurysms (CAAs). These CAAs can result in cardiac complications (1) and even be fatal when an occlusion occurs due to secondary complications such as thrombosis or stenosis (2). If treated properly, i.e., preferably within the first 10 days of illness with intravenous immunoglobulin (IVIG) and oral aspirin for a prolonged period of time, a decrease in the incidence of CAAs has been reported (3, 4). However, with this treatment still ∼2.4–27.8% of the KD patients develop CAAs (5–11). To further prevent the development of CAAs due to KD, we need more understanding of the etiology of the disease. Multiple studies suggest that both infectious and genetic factors are involved (12–14). Most likely KD is triggered in genetically pre-disposed children by an intracellular pathogen (15). Several genes have been identified to be associated with KD in children (e.g., ITPKC, CASP3, CD40, and FCGR2A) (16–30). Possible genetic contribution

to the development of CAAs and the presence of so-called therapy or IVIG resistance is also a topic of research (30–40).

Over the past decades research also aimed to find a singular ubiquitous pathogen, that could trigger the immune system in genetically pre-disposed children. The hypothesis of an infectious pathogen is supported by the epidemiologic features of KD, namely that young infants under the age of 6 months and adults very rarely develop KD (12, 41, 42). Young infants are protected by maternal antibodies, and adults already would have been exposed to this pathogen and protected by the adaptive immune system. Moreover, sudden peaks in incidence as observed in Japan, support such singular transmissible agents to evoke KD (43, 44). The current COVID-19 pandemic (caused by coronavirus SARS-Cov-2) also suggests that an infectious agent can, at least in some of the affected children, cause a syndrome similar or even identical with KD or KD-like shock syndrome (45–48). Despite extensive research, a common pathogen as the cause of KD has not been identified and a variety of pathogens have been found in KD patients in the past such as SARS-CoV-2, measles, human herpesvirus-6, parvovirus B19 and EBV (45, 49–55). Most likely the immune response causing KD can be triggered by multiple pathogens, as opposed to one single and specific pathogen.

Studies have suggested that the immune response in KD is mainly IgA-driven with a notable absence of IgM and IgG at the affected sites (41, 56, 57). Also high concentrations of IgA have been found at mucosal sites of the trachea of KD patients (56). These findings suggest that the upper respiratory tract could be the point of access for the pathogen to enter the body and consequently triggering KD, which may be associated with the local enlargement of often unilateral lymph nodes in the sternocleidomastoideus region. Studies relating wind patterns to the spread of KD further support the suggestion of an air-borne factor (58, 59). The higher prevalence of KD among Japanese, Taiwanese and children from South-East Asia as well as the familial cases and incidence among identical twins suggest a genetic risk, which suggest that an infectious trigger causes KD in genetically susceptible individuals. The genetic impact and its contribution to the risk to develop KD seems limited since the disease is particularly prevalent under the age of 5 (11). This suggests that development of immune reactivity plays an important role in the inherent susceptibility to the often undefined KD triggers.

An under-challenged immune system at an early age has previously been proven to pre-dispose children to specific diseases such as Acute Lymphoblastic Leukemia (60). This abnormal response to a common infection due to an under-challenged immune system has become known as the "Greaves" hypothesis (61, 62). According to this theory, a low exposure to common infections at a young age, could influence the development of the immune system and result in an altered development and maturation of the adaptive immune system. This would suggest that in the case of KD, not only nature (genetic factor) plays an important role in the pre-disposition of KD but also nurture (degree of exposure to common infections or environmental immune-reactive substances due to e.g., hygiene measures or living conditions) could play an important role to develop the disease at early age. A low exposure could facilitate a pathological response to a pathogen and triggering KD.

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are both highly contagious viruses, which are commonly acquired during early childhood, and remain systemically suppressed in healthy individuals but ever-present, hence shaping or imprinting the immune reactivity of a given individual (63). CMV and EBV are a good reflection of viral exposure, as they spread through bodily fluids, as other commonly acquired viruses do (such as influenza, parvovirus etc.). Therefore, we used CMV and EBV as an indicator of the exposure state to systemic pathogens, reasoning that ever-present viruses would be beneficial to prepare the immune reactivity to subsequent infections. The aim of this study was to assess whether the incidence of CMV and EBV infection is lower in children with KD compared to a control pediatric population as a potential read-out for the under-challenged state of the immune system in those children affected by KD.

## MATERIALS AND METHODS

### Patient Population

Based on the criteria of the American Heart Association (AHA) (64), patients with KD from the Dutch national referral center for KD were included. Retrospectively, we collected CMV and EBV antibody test results [IgG to EBNA (Epstein Barr nuclear antigen), IgG and IgM to EBV VCA (viral capsid antigen), and IgG and IgM to CMV] during follow up. After we excluded the patients with positive IgM to exclude any acute infections and the risk of cross-reactive serology results of EBV and CMV, we calculated seroprevalence. Clinical information was extracted from medical records. The blood samples taken within 9 months of IVIG treatment (or 10 months in case of a second IVIG treatment within a month of first treatment) were excluded. Samples taken later than 2 years after onset of disease and patients that were treated for a second episode of KD were excluded. Samples taken at the time of diagnosis, prior to IVIG infusion were included. We categorized the results according to age and we defined the following age groups: 0.5–2 years, 2–4 years, 4–6 years, 6–10 years, and 10–18 years.

### Control Group

We compared the age-dependent CMV- and EBV-seroprevalence in our KD patients to CMV- and EBV-seroprevalences in a control group. For this control group we used the requested EBV VCA IgG and IgM, as well as CMV IgG and IgM results (in the age group 6 months until 18 years) from the same laboratory as the one used by the national referral center from January 2000 until June 2019. As our hospital is a national referral center for KD, the follow-up of KD patients takes place exclusively within our KD team, therefore we were able to exclude KD patients from the control group by simply disqualifying the EBV VCA IgG and CMV IgG requested by our KD team members (physicians

**Abbreviations:** CAAs, Coronary artery aneurysms; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; VZV, varicella-zoster virus.

**TABLE 1 |** Age-matched IgG seropositivity for CMV (and/or EBV anti-VCA) in KD cases vs. controls.

| Age at time of measurement (years) | CMV+ | | | | | EBV+ | | | | | Both EBV+ and CMV+ at sampling | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KD | | No KD | | *P*- value | KD | | No KD | | *P*-value | KD | | No KD | | *P*-value |
| | *n* | % | *n* | % | | *n* | % | *n* | % | | *n* | % | *n* | % | |
| 0.5–2 | 1/15 | 6.7 | 259/716 | 36.2 | 0.018 | 2/14 | 14.3 | 190/679 | 28.0 | 0.257 | 0/12 | 0.0 | 59/476 | 12.4 | 0.193* |
| 2–4 | 6/28 | 21.4 | 322/877 | 36.7 | 0.098 | 6/29 | 20.7 | 410/934 | 43.9 | 0.013 | 4/27 | 14.8 | 142/691 | 20.5 | 0.468* |
| 4–6 | 5/19 | 26.3 | 279/796 | 35.1 | 0.430 | 3/17 | 17.6 | 495/954 | 51.9 | 0.005 | 1/17 | 5.9 | 146/644 | 22.7 | 0.100 |
| 6–10 | 5/15 | 33.3 | 492/1202 | 40.9 | 0.552* | 4/16 | 25.0 | 840/1406 | 59.7 | 0.005 | 1/15 | 6.7 | 275/986 | 28.4 | 0.063 |

*Not enough power.

treating KD patients). We also excluded children from the control group with a positive IgM for CMV and/or EBV. Only the first requested CMV and EBV sample of a patient was included. If a laboratory result turned out to be inconclusive (borderline value or not enough blood drawn) this result was excluded, and a possible subsequent value was included instead. "Weak positive" results were regarded as positive. Finally we categorized the results according to age in the same categories as the KD group.

## Laboratory Analysis

Following the instructions of the manufacturers, IgG antibodies to EBV initially were determined using the anti-EBV VCA IgG, and EBNA IgG ELISAs from Biotest/BioRad Medical Diagnostics GmbH (Dreieich, Germany); later using the automated Liaison assays from DiaSorin (Saluggia, Italy); IgG to CMV initially was determined using the automated AxSym assays from Abbott Diagnostics (Chicago, USA); later using the automated Liaison assays from DiaSorin (Saluggia, Italy).

## Statistical Analysis

Our data consisted of a small sample size with a binomial distribution, therefore we performed a chi-squared test of independence using IBM Corp. Released 2016 SPSS Statistics for Windows, Version 24.0 to compare the seroprevalences in the Kawasaki group with their age- peers in the control group.

# RESULTS

As shown in **Table 1**, the prevalence of CMV IgG was 6.7% in the KD group as compared to 36.2% in the control group in the 0.5–2 years age category; 21.4% compared to 36.7% in the 2–4 years old category; 26.3% compared to 35.1% in the 4–6 age group and 33.3% compared to 40.9% in the 6–10 years old category. *P*-values were 0.018, 0.098, 0.430, and 0.552, respectively. Similarly we found a lower prevalence of EBV VCA IgG in the KD group as compared to the control group i.e., 14.3% as compared to 28.0% in the age category 0.5–2 years; 20.7% compared to 43.9% in the 2–4 years old category; 17.6% compared to 51.9% in the 4–6 years old category and 25.0% compared to 59.7% in the 6–10 years old category. *P*-values were 0.257, 0,013, 0.005, and 0.005, respectively. When combined we also found a lower seroprevalence of CMV and EBV together, in the KD group compared to the control group, but these differences did not reach levels of statistical significance.

From the 855 KD patients in our database 585 patients had CMV IgG and 586 patients had EBV VCA IgG determined during follow-up. From these patients, 86 patients had CMV IgG and another 86 patients had EBV VCA IgG determined within our chosen timeframe (**Figure 1**). The majority of the CMV IgG-tested KD patients (67.4%) was male and 8.1% had a partially reported Asian ethnicity. Similarly the majority of the EBV VCA IgG KD tested patients was male (68.6%) and 8.1% had a parental report of a mixed, partial Asian ethnicity (**Table 2**).

After categorizing the age groups, there was insufficient data of KD children in the age category of >10 years, therefore we did not use this age group for our comparison. In the KD patients with CMV IgG measurements, 12 patients did not receive IVIG and 12 patients in whom IVIG was administered, the CMV IgG measurement took place at diagnosis of KD prior to IVIG (**Table 3**).

Since we know that seroreactivity after administration of IVIG may last for more than 6–9 months (65) we assessed the median time that had passed between administration of IVIG and the measurement of the seroresponse of CMV IgG in the remaining KD patients which was 14 months (range 0–23) (**Table 3**). In the KD patients with EBV VCA IgG measurements, 11 patients did not receive IVIG and in 11 patients the EBV VCA IgG measurement took place at KD diagnosis prior to IVIG (**Table 3**). Also in the remainder of this group the median time that had passed between administration of IVIG and measurement of the seroresponse of EBV VCA IgG was 14 months (range 0–23).

For the control group, a total of 3114 EBV VCA IgG samples and 3591 CMV IgG samples were analyzed by the laboratory of by the national referral center. By comparison, the prevalence of CMV and EBV was lower in the KD population in all age groups tested against their age-matched peers of the control group (**Figures 2A–C**).

# DISCUSSION

In the KD population, the prevalence of past CMV and EBV infection as indicated by seroprevalence, is lower than in an age-matched control group. This suggests that prior exposure to systemic infections by common viruses such as CMV and

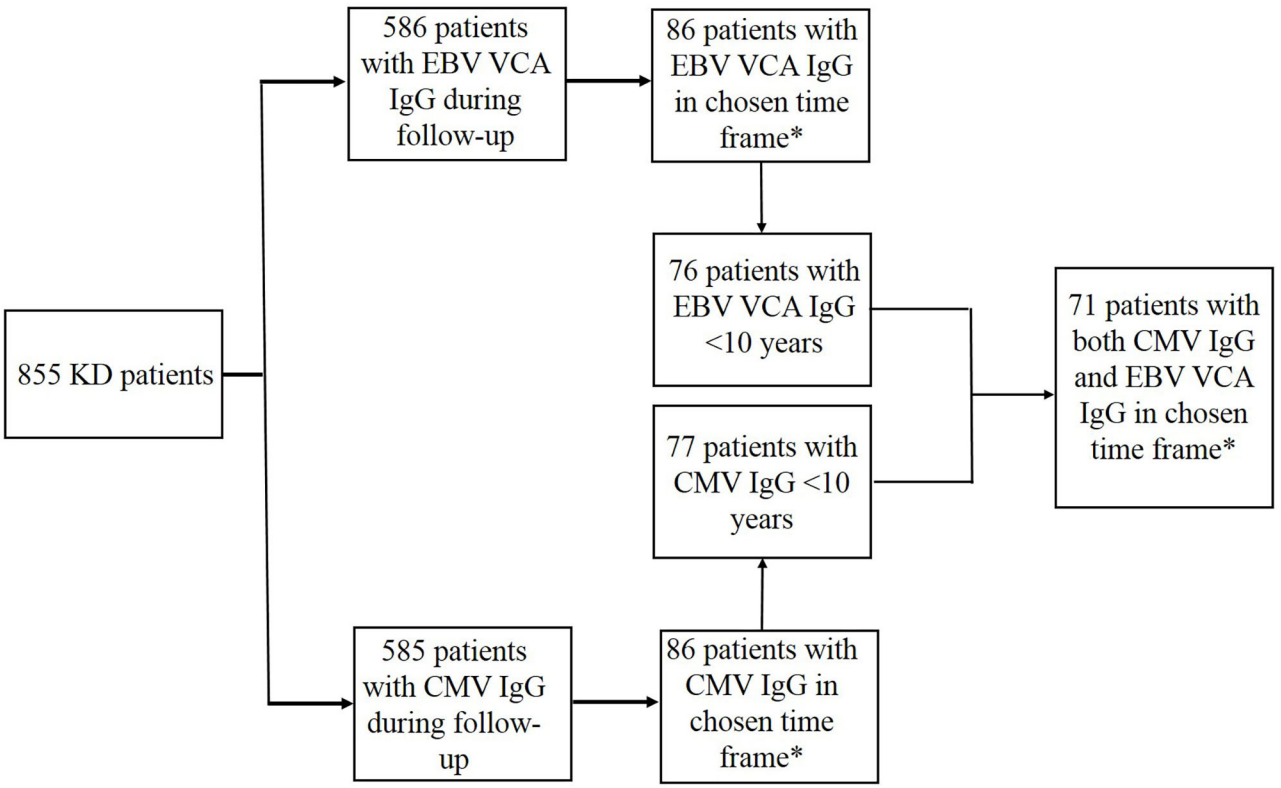


**FIGURE 1 |** Patient selection. *Chosen time frame was 9 months after IVIG treatment but not later than 2 years after onset of disease.

EBV might have a protective role in the susceptibility to KD at young age.

In the past, low exposure to infectious agents in early childhood has been proposed to increase the susceptibility to allergic diseases and autoimmune diseases due to the influence on the development of the immune system (66). Allergic reactions are known to be initiated by T helper lymphocytes while in KD a cellular immune dysfunction i.a. the imbalance of Th1 and Th2 or "split T cell anergy" has been suggested (67, 68). The theories about hygiene or the under-challenged immune system due to low exposure to environmental or infectious agents, have been suggested to contribute to a pathological response to a pathogen (69), which may be relevant to triggering KD in genetically susceptible children. Several studies have shown an increase of allergies in KD patients compared to the general population, which may support a link between hygiene and environmental conditions involved in the slow but progressive rise in KD incidence worldwide which can be neither caused by improved recognition and diagnostic skills of doctors nor by current laboratory tests for KD since these are not yet applicable (70–73).

An under-challenged immune system has previously been reported to have a causal connection to pre-dispose children to specific diseases such as Acute Lymphoblastic Leukemia (60, 61, 64). However, the majority of these studies used daycare attendance as an indicator for exposure to common infections, which is a very indirect measurement. Reasoning that at young age a viral infection could be such a trigger knowing that in the past several viral infections have been suggested as the cause of KD (49–54), we used CMV and EBV IgG seropositivity as an indication for exposure to common infections in this study. By excluding IgM positive results, we excluded the possibility of cross-reactive antibody results, which are a possible confounding factor if you look at an acute infection of these two viruses. Once infected, herpes viruses such as CMV and EBV stay lifelong present and do not cause any symptoms in healthy individuals, being suppressed by the immune system (63, 74, 75). Viral reactivation of herpes viruses may sometimes occur, especially during moments of reduced T-cell mediated immune suppression by concurrent disease such as malignancy, chronic disease, or the use of immunosuppressive medication, which then causes a raised alertness of the immune system against these viruses to keep them constrained. If immunity cannot suppress these reactivated viruses, the reactivated viruses may result in clinical disease. We have not observed any clinical reactivation as indicated by shingles or herpes zoster by herpes simplex virus (HSV) or varicella-zoster virus (VZV), respectively, or EBV or CMV DNA plasma concentrations during acute KD, indicating a globally intact immunity in these children.

When measuring seroprevalence of EBV and CMV, we observed a significant difference for EBV VCA IgG exposure in the KD group compared to the control group, except for the very young age category of 0.5–2 years old. The lack of significance

**TABLE 2 |** Characteristics KD population.

| | CMV IgG tested | | EBV VCA IgG tested | | Both EBV VCA IgG and CMV IgG tested on the same date | |
|---|---|---|---|---|---|---|
| | ***n*** **(total = 86)** | **%** | ***n*** **(total = 86)** | **%** | ***n*** **(total = 77)** | **%** |
| **IVIG treatment** | | | | | | |
| 0.5–2 years | 12 (15) | 80.0 | 12 (14) | 85.7 | 10 (12) | 83.3 |
| 2–4 years | 25 (28) | 89.2 | 26 (29) | 89.7 | 25 (27) | 92.6 |
| 4–6 years | 15 (19) | 78.9 | 13 (17) | 76.5 | 13 (17) | 76.5 |
| 6–10 years | 12 (15) | 80.0 | 12 (16) | 75.0 | 12 (15) | 80.0 |
| 10–18 years | 6 (9) | 66.7 | 8 (10) | 80.0 | 5 (6) | 83.3 |
| **Male gender** | | | | | | |
| 0.5–2 years | 10 (15) | 66.7 | 9 (14) | 64.3 | 8 (12) | 66.7 |
| 2–4 years | 18 (28) | 64.3 | 19 (29) | 65.5 | 17 (27) | 63.0 |
| 4–6 years | 14 (19) | 73.7 | 13 (17) | 76.5 | 13 (17) | 76.5 |
| 6–10 years | 8 (15) | 53.3 | 9 (16) | 56.3 | 8 (15) | 53.3 |
| 10–18 years | 7 (9) | 77.8 | 9 (10) | 90.0 | 6 (6) | 100 |
| **Complete KD*** | | | | | | |
| 0.5–2 years | 8 (15) | 53.3 | 8 (14) | 57.1 | 6 (12) | 50.0 |
| 2–4 years | 22 (28) | 78.6 | 21 (29) | 72.4 | 21 (27) | 77.8 |
| 4–6 years | 14 (19) | 73.7 | 13 (17) | 76.5 | 13 (17) | 76.5 |
| 6–10 years | 12 (15) | 80.0 | 13 (16) | 81.3 | 13 (15) | 86.7 |
| 10–18 years | 6 (9) | 66.7 | 9 (10) | 90.0 | 5 (6) | 83.3 |
| **No enlargement of coronary artery in acute phase (*Z* score < 2.5)**** | | | | | | |
| 0.5–2 years | 10 (15) | 66.7 | 9 (14) | 64.3 | 6 (12) | 50.0 |
| 2–4 years | 19 (28) | 67.9 | 20 (29) | 68.9 | 18 (27) | 66.7 |
| 4–6 years | 14 (19) | 73.7 | 12 (17) | 70.5 | 12 (17) | 70.6 |
| 6–10 years | 11 (15) | 73.3 | 12 (16) | 75.0 | 11 (15) | 73.3 |
| 10–18 years | 5 (9) | 55.6 | 6 (10) | 60.0 | 4 (6) | 66.7 |
| **Enlargement of coronary artery in acute phase (*Z* score 2.5–10)**** | | | | | | |
| 0.5–2 years | 3 (15) | 20.0 | 3 (14) | 21.4 | 3 (12) | 25.0 |
| 2–4 years | 2 (28) | 7.1 | 2 (29) | 10.5 | 2 (27) | 7.4 |
| 4–6 years | 3 (19) | 15.8 | 3 (17) | 17.6 | 3 (17) | 17.6 |
| 6–10 years | 1 (15) | 6.7 | 1 (16) | 6.25 | 1 (15) | 6.7 |
| 10–18 years | 1 (9) | 11.1 | 1 (10) | 10.0 | 1 (6) | 16.7 |
| **Giant aneurysm in acute phase (*Z* score ≥ 10)**** | | | | | | |
| 0.5–2 years | 2 (15) | 13.3 | 2 (14) | 14.3 | 2 (12) | 16.7 |
| 2–4 years | 4 (28) | 14.3 | 4 (29) | 13.8 | 4 (27) | 14.8 |
| 4–6 years | 1 (19) | 5.3 | 1 (17) | 5.9 | 1 (17) | 5.9 |
| 6–10 years | 0 (15) | 0 | 0 (16) | 0 | 0 (15) | 0 |
| 10–18 years | 0 (9) | 0 | 0 (10) | 0 | 0 (6) | 0 |
| **Dutch ethnicity*** (both parents)** | | | | | | |
| 0.5–2 years | 7 (15) | 46.7 | 6 (14) | 42.9 | 4 (12) | 33.3 |
| 2–4 years | 13 (28) | 46.4 | 13 (29) | 44.8 | 13 (27) | 48.1 |
| 4–6 years | 8 (19) | 42.1 | 8 (17) | 47.1 | 8 (17) | 47.1 |
| 6–10 years | 7 (15) | 46.7 | 8 (16) | 50.0 | 7 (15) | 46.7 |
| 10–18 years | 6 (9) | 66.7 | 7 (10) | 70.0 | 4 (6) | 66.7 |
| **(Partially) Asian ethnicity***, **** | | | | | | |
| 0.5–2 years | 0 (15) | 0 | 0 (14) | 0 | 0 (12) | 0 |
| 2–4 years | 3 (28) | 10.7 | 3 (29) | 10.3 | 3 (27) | 11.1 |
| 4–6 years | 2 (19) | 10.5 | 2 (17) | 11.8 | 2 (17) | 11.8 |
| 6–10 years | 2 (15) | 13.3 | 2 (16) | 12.5 | 2 (15) | 13.3 |
| 10–18 years | 0 (9) | 0 | 0 (10) | 0 | 0 (6) | 0 |

*(Continued)*

**TABLE 2 |** Continued

| | CMV IgG tested | | EBV VCA IgG tested | | Both EBV VCA IgG and CMV IgG tested on the same date | |
|---|---|---|---|---|---|---|
| | ***n* (total = 86)** | **%** | ***n* (total = 86)** | **%** | ***n* (total = 77)** | **%** |
| **Other or unknown ethnicity*** ** | | | | | | |
| 0.5–2 years | 8 (15) | 53.3 | 8 (14) | 57.1 | 8 (12) | 66.7 |
| 2–4 years | 12 (28) | 42.9 | 13 (29) | 44.8 | 11 (27) | 40.7 |
| 4–6 years | 9 (19) | 47.4 | 7 (17) | 41.2 | 7 (17) | 41.2 |
| 6–10 years | 6 (15) | 40.0 | 6 (16) | 37.5 | 6 (15) | 40.0 |
| 10–18 years | 3 (9) | 33.3 | 3 (10) | 30.0 | 2 (6) | 33.3 |

**Complete KD is defined as: fever, combined with a minimum of four of the five other symptoms (rash, conjunctivitis, lymphadenopathy, changes of extremities and changes of the mouth). In 4 patients the symptoms were unknown.*
***Z score was calculated using the McCrindle/Boston model.*
****Ethnicities were reported by parents/care-givers.*
*****Asian countries that were included were: Vietnam, Japan, China, Indonesia and Thailand.*

**TABLE 3 |** Time between administration of IVIG and measurement of seroresponse (Δ Time).

| | **Δ Time CMV IgG (median, range) in months** | **Δ Time EBV VCA IgG (median, range) in months** |
|---|---|---|
| **0.5–2 years** | 14 (12–17), $n = 8$ | 14 (12–16), $n = 9$ |
| Patients with measurement at diagnosis, prior to IVIG | $n = 4$ | $n = 3$ |
| Patients that did not receive IVIG | $n = 3$ | $n = 2$ |
| **2–4 years** | 15 (10–22), $n = 21$ | 15 (10–22), $n = 21$ |
| Patients with measurement at diagnosis, prior to IVIG | $n = 4$ | $n = 5$ |
| Patients that did not receive IVIG | $n = 3$ | $n = 3$ |
| **4–6 years** | 14 (10–23), $n = 13$ | 14 (10–23), $n = 12$ |
| Patients with measurement at diagnosis, prior to IVIG | $n = 3$ | $n = 2$ |
| Patients that did not receive IVIG | $n = 3$ | $n = 3$ |
| **6–10 years** | 13 (10–22), $n = 11$ | 14.5 (10–22), $n = 12$ |
| Patients with measurement at diagnosis, prior to IVIG | $n = 1$ | $n = 1$ |
| Patients that did not receive IVIG | $n = 3$ | $n = 3$ |

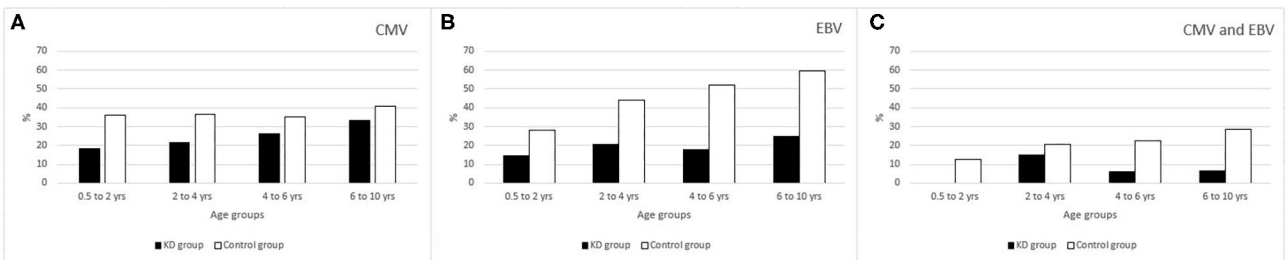


**FIGURE 2 | (A)** % of CMV seropositivity. **(B)** % of EBV seropositivity. **(C)** % of CMV and EBV combined seropositivity.

could be explained by a generally low exposure to EBV in this age group and hence an underpowered comparison. In the same age group of 0.5–2 years old, there was a noticeable difference for CMV IgG seropositivity, which could be explained by higher contagiousness of CMV in early childhood and unnoticed transmission by breastfeeding, which may transmit CMV but far less commonly EBV (76–82). The differences in seroprevalance between EBV and CMV seem to decrease as age increases in our KD cohort. Of the positive EBV anti-VCA IgG measurements a few had anti-EBNA measurements ($n = 12$). Anti-EBNA antibodies are usually undetectable during the (sub)acute phase of the infection and slowly appear after onset of disease within the following weeks or months. Therefore, a positive anti-EBNA response indicates an infection in the past. From the anti-EBNA

measurements most were negative (67%), with a median time of 14.5 months (range 11–18 months) between IVIG infusion and date of measurement. These infections could therefore be more recent, possibly after KD. But caution needs to be taken with the interpretation of anti-EBNA measurements due to the wide range in which even healthy controls can become positive. Of the cases in which both anti-EBNA and anti-VCA IgG were positive, the median time between IVIG and date of measurement was 14.5 months (range 11–18 months). We did not assess the anti-EBNA measurements in the control group due to the before mentioned limited value of these EBNA antibodies (83). The possibility of spurious IgG seropositive measurements because of prior IVIG infusion (65, 84) was excluded because of the time passed [14 months (range 0–23)] between the administration of IVIG and the measurement of the actual serology.

CMV is known to challenge the immune system, altering the immune response to other pathogens (85). Both EBV and CMV cause a redistribution in B-cell subsets (86–88). Herpes viruses like EBV and CMV causing systemic infection of the immune system and the lifelong survival within the host with transient virus reactivation from time to time—going most often completely unnoticed and without any clinical symptoms (74, 89–96) –, are expected to leave an imprint on the immune system because of their lifelong infection of that same system. This effect on the host's immune system could affect the response to a pathogen, also to the as yet unidentified and probably miscellaneous agents that may trigger KD. Although we cannot directly prove the hypothesis, our findings may suggest that insufficient priming of the immune system at an early age plays a role in the immune response in KD. Although we do not want to overemphasize a direct role in KD susceptibility for the two herpesviruses that we have tested, the rising incidence of KD in Japan, and the falling incidence of EBV at the same time (97) would support this theory on an under-challenged immune system. At the molecular level the immunological data currently available on epigenetic imprinting of T and B lymphocytes also indicate that previous infections impact the reactivity against subsequent triggers. These phenomena are definitely not considered as KD-specific or selective for certain age categories, but it supports the notion that prior exposure shapes the immunological outcome. Similar findings have been demonstrated at the epigenetic level of the innate immune system by exposure to mycobacterial components, which more recently has been a basis for BCG immunization to protect from Sars-CoV2 infection, as an example of "trained (innate) immunity" (98). The induction of a mature and experienced immune system to protect from a wide variety of triggers at young age may help to understand the otherwise unexplained incidence of KD in the under-5 years old while becoming almost zero at late adolescence (99, 100).

This is a study with intrinsic limitations, namely our control group might not be a representation of a completely healthy population due to the fact that the serological tests were requested by general practitioners and doctors from the hospital for KD-unrelated reasons. On the other hand, these anonymous controls were screened for infection similar to the children presenting with KD, for that reason being a cohort that might be more comparable with our KD children than a completely healthy population.

## CONCLUSION

We observed a significantly reduced CMV and EBV prevalence in KD patients compared to age-matched controls. The data suggest that an under-challenged immune system may contribute to the inflammatory vasculitis in children with an inherent polygenic and complex susceptibility to KD.

## DATA AVAILABILITY STATEMENT

The original contributions generated in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

DS conceptualized the study, collected data, interpreted data, and drafted the manuscript. AS collected data, performed the analysis, and contributed in the initial draft of the manuscript. TK and HZ coordinated and supervised data collection and reviewed the manuscript for important intellectual content and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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# CT Angiography or Cardiac MRI for Detection of Coronary Artery Aneurysms in Kawasaki Disease

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**Background:** Kawasaki disease (KD) is an acute vasculitis that mainly affects the coronary arteries. This inflammation can cause coronary artery aneurysms (CAAs). Patients with KD need cardiac assessment for risk stratification for the development of myocardial ischemia, based on *Z*-score (luminal diameter of the coronary artery corrected for body surface area). Echocardiography is the primary imaging modality in KD but has several important limitations. Coronary computed tomographic angiography (cCTA) and Cardiac MRI (CMR) are non-invasive imaging modalities and of additional value for assessment of CAAs with a high diagnostic yield. The objective of this single center, retrospective study is to explore the diagnostic potential of coronary artery assessment of cCTA vs. CMR in children with KD.

**Methods and Results:** Out of 965 KD patients from our database, a total of 111 cCTAs (104 patients) and 311 CMR (225 patients) have been performed since 2010. For comparison, we identified 54 KD patients who had undergone both cCTA and CMR. CMR only identified eight patients with CAAs compared to 14 patients by cCTA. CMR missed 50% of the CAAs identified by cCTA.

**Conclusions:** Our single center study demonstrates that cCTA may be a more sensitive diagnostic tool to detect CAAs in KD patients, compared to CMR.

**Keywords: Kawasaki disease, imaging, cardiac MRI, coronary computed tomographic angiography, coronary artery aneurysms, coronary artery assessment**

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## INTRODUCTION

Kawasaki disease (KD) is an acute vasculitis of the medium-and-small-sized arteries of unknown etiology. To date, KD is the most common acquired pediatric heart disease in Western society (1). The vasculitis mainly affects the coronary arteries and the inflammation can cause coronary artery aneurysms (CAAs). Due to the formation of CAAs, CAA-related secondary complications can occur such as thrombosis, calcification, and stenosis/occlusion which can lead to myocardial ischemia. The occurrence of stenosis and thrombosis may well be inherent to the size of the CAA (2). Currently, echocardiography is used as the primary imaging modality in KD, and is a good first, rapid, and non-invasive screening tool in the acute phase. According to the American Heart Association (AHA) guidelines of 2017, additional imaging should be considered

during follow-up after the patient has been categorized (by echocardiography) with a CAA (Z-score $\geq$ 2.5) (3), due to an associated increased risk for myocardial ischemia. This is where the Japanese Circulation Society (JCS) differs in their recommendations from the AHA. Low diagnostic accuracy of echocardiography due to limited visualization of distal coronary segments may result in underestimation of the CAA burden and may increase the risk for secondary complications (4). Therefore, the JCS suggests performing additional imaging in the convalescent phase (5) for a more accurate categorization of CAA severity.

Invasive Coronary Angiography (CAG), coronary computed tomographic angiography (cCTA) and Cardiac MRI (CMR) have been suggested as alternatives complementary to echocardiography by the AHA guidelines of 2017. Invasive CAG is not routinely used because of its invasive nature and risk of complications. In a recent overview, the need for guidance for the long-term management of KD patients was emphasized, suggesting non-invasive modalities such as echocardiography and CMR, and only when other modalities cannot be used, to consider low radiation dose computed tomography (CT) (6). As the limitations of echocardiography are known, we have been performing additional imaging such as cCTA and CMR, in selected patients in our national referral center (7). In our previous study we have demonstrated the relevance of routine additional imaging for coronary artery assessment by evaluating echocardiography and cCTA for the detection of CAAs, secondary coronary artery pathology, and radiation exposure (4). In the current study, we took the approach to investigate CAA detection with cCTA and CMR, if imaging results were both available in the same patient during follow-up. The aim of this retrospective single-center study is to compare the diagnostic yield of cCTA and CMR in clinical practice for the detection of CAAs in KD patients.

# METHODS

## Study Population

Patients that met the AHA diagnostic criteria for KD, and presented to the follow-up of the national referral center for KD in the Netherlands and underwent CMR and cCTA between the year 2008 and 2020, were retrospectively included in this study. The AHA diagnostic criteria for KD are: persistent fever for $\geq$5 days and $\geq$4 of the five clinical features (rash, conjunctivitis, cervical lymphadenopathy, oral changes, and extremity changes) in the case of complete KD and, for incomplete KD, if fewer than 4 of the clinical features with prolonged unexplained fever and compatible echocardiography and/or laboratory findings are present. As cCTA and CMR are not part of the routine cardiac assessment in patients with KD, these patients are a selected subset of the KD population. The majority of these patients had been previously diagnosed with CAA upon echocardiography. It is exactly this group of patients (with proximal coronary artery pathology upon echocardiography) that has the risk for potentially missed distal coronary artery involvement upon echocardiography, since the patients with no proximal involvement have no reports on having distal involvement (4). The primary objectives for additional imaging in these patients were: i.e., to verify whether CAAs could be missed in the distal parts of the coronary arteries, beyond the window of inspection by echocardiography, and—at the same time—to look for secondary coronary artery pathology (such as stenosis, occlusion, calcification, or thrombus formation). Beta-blockers were used if patients were older than the age of 12 and had a heart rate above 70–75 beats per minute (BPM) prior to the imaging. Clinical information about the acute phase and of the follow-up was extracted from medical records. Institutional Review Board approval was obtained.

**Abbreviations:** AHA, American Heart Association; ASA, acetylsalicylic acid; BPM, beats per minute; CAAs, Coronary artery aneurysms; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CAG, coronary angiography; cCTA, coronary computed tomographic angiography; CMR, cardiac magnetic resonance imaging; CT, computed tomography; Cx, circumflex; IVIG, intravenous immunoglobulin; JCS, Japanese Circulation Society; KD, Kawasaki disease; LAD, left anterior descending artery; LMCA, left main coronary artery; MRA, magnetic resonance coronary angiography; MRI, magnetic resonance imaging; RCA, right coronary artery; TE, echo time; TR, repetition time.

## CMR

Magnetic resonance imaging (MRI) images were acquired using a 1.5-T whole body MRI scanner with cardiac software (Siemens, Magnetom, Avanto; Siemens, Erlangen, and Germany). The imaging protocol included a navigator gated, ECG-triggered, non-contrast enhanced magnetic resonance coronary angiography (MRA) series, using a 3D echo time (TE)/repetition time (TR) optimized steady state free precession sequence with a fat saturated prepulse and T2 preparation (FOV 340–400 mm, base resolution 288 pixels. This resulted in a 3D image with a resolution of $\sim$0.6 $\times$ 0.6 $\times$ 1.0 mm/pixel, encompassing the entire coronary tree. Acceptance window of the navigator was set to 2 mm. ECG triggering was set to the period of diastasis in the heart cycle. Imaging results were discussed in a multi-disciplinary team, consisting of a radiologist, cardiologist, pediatric cardiologist, and pediatric immunologist (all with expertise in KD).

## cCTA

For cCTA a dual-source 2 $\times$ 192-slice multidetector CT scanner (Siemens Somatom Force, Erlangen, Germany) was used from November 2015. Before 2015, the cCTA images were acquired using a 64-slice CT scanner (Philips, Brilliance64). For both scanners a prospective ECG-triggered step-and-shoot protocol was used and images were reconstructed with a slice-thickness of 0.6 and 0.9 mm, respectively. Contrast medium (Ultravist 300 mg/ml, Bayer Healthcare Pharmaceuticals) was administered intravenously. The total iodine dose and iodine delivery rate were adjusted for body weight. The scan delay was determined using a test bolus, after which 4 s were added for the scan delay of the main bolus. A multidisciplinary team including a radiologist, cardiologist, pediatric cardiologist, and pediatric immunologist (with expertise in KD) discussed the results, as reported previously (4).

## Measurements

Coronary artery diameters were measured and the CAAs *Z*-score was calculated according to the McCrindle/Boston model (8). There is no current alternative to the standardized measures of CAA obtained by echocardiography (9). Hence, we have used these values as the best available substrate, recognizing that they are obtained by measuring different components of the coronary arteries (internal appearance of wall to wall on echocardiography, rather than luminal diameter of contract on cCTA/CMR). A *Z*-score $\geq$ 3 was considered to be an aneurysm (as compared to a *Z*-score $\geq$ 2.5, which is normally used). By using a cut-off of a *Z*-score $\geq$ 3 instead of a *Z*-score $\geq$ 2.5 we aimed to increase specificity because a *Z*-score $\geq$ 2.5 in 1 coronary artery branch occurs in 0.6% of afebrile children and a Z-score $\geq$ 3.0 occurs in 0.1% (3). Also a study found that coronary artery dimensions in febrile children (non-KD) are larger than those in afebrile children, but smaller than in febrile KD patients (9). Even though not performed at the same age, majority of the imaging took place in the stable phase of the disease (i.e., more than 2 years after onset of disease) when remodeling is not expected anymore. Thereafter, discrepancies in outcome (number of detected CAAs) were considered the result of a lack of diagnostic accuracy. The left main coronary artery (LMCA), left anterior descending artery (LAD), right coronary artery (RCA), and circumflex (Cx) were evaluated. A CAA in the Cx was defined as luminal diameter $\geq$ 4.0 mm (10). Not only luminal dimensions were visualized, also myocardial ischemia, vascular stenosis, occlusion, vessel wall calcification and intravascular thrombosis were reported. Stenosis was defined as a narrowed lumen which influences the blood flow while an occlusion is a complete blockage of the lumen with no reserve flow. When on CMR, the coronary arteries were not visualized distinctly enough to make accurate and reliable measurements, coronary arteries were classified as NORMAL/ABNORMAL by two independent radiologists, blinded for the initial echocardiography and any additional imaging.

## Statistics

We generated demographic characteristics of KD patients who underwent both cCTA and CMR, presented as numbers with percentages and, where appropriate, with their mean or median and ranges (**Table 1**).

**TABLE 1 |** Demographics and characteristics of 54 KD patients with imaging performed both by cCTA and CMR.

| Demographics | *n* = 54 | Remarks |
|---|---|---|
| Male | *n* = 43 | |
| Female | *n* = 11 | |
| Age in years at onset KD (median, range) | 3.1 (0.12–11.15) | Age-at-onset was unknown in two patients. |
| Missed diagnosis, no treatment | *n* = 7 | No treatment (IVIG/prednisone) received in seven cases, of which two did receive ASA. |
| Day of treatment after onset of fever (median, range) | 8 (4–26) | In 2 patients the day of treatment was unclear. |
| Treatment > 10 days after fever onset | *n* = 11 | |
| Non-responder to 1st IVIG | *n* = 11 | Persistent fever > 48 h after IVIG treatment. |
| ΔTime in years (time between CMR and cCTA) (median, range) | 3 (0–7) | In 3 patients the cCTA was performed after the CMR. ΔTime in years (time between cCTA and CMR) for these patients was 1, 1, and 4 years. |
| **CAA *Z*-score acute stage** | | |
| • *Z*-score > 10* (giant) | *n* = 12 | |
| • *Z*-score 3–10* (small- to medium-sized aneurysms) | *n* = 13 | |
| • *Z*-score < 3* (no aneurysm) | *n* = 23 | |
| • Unknown | *n* = 6 | |

**CAA status is based on prior echocardiography results in the acute phase of KD.*

# RESULTS

## Study Population

We collected the cCTA and CMR results from 54 pediatric KD patients who had undergone both imaging techniques during the follow-up and we compared the imaging results retrospectively, of which nine were performed before 2015. All of the CMRs were executed prior to cCTA, except for three cases. The majority of the study population in which both cCTA and CMR scanning has been performed, was male (80%). The median age at onset of disease was 3.1 years (range 0.12–11.15). A total of 12 patients (22%) had giant aneurysms (*Z*-score $\geq$ 10) following the acute presentation with clinical KD (**Table 1**). Classic KD diagnosis presenting with $\geq$4 of the 5 principal clinical features was present in the majority of cases (74%), incomplete KD was present in a minority (20%), and in the remaining three patients (6%) the clinical features at the acute stage of the disease were unknown. Most patients had been treated adequately with oral acetylsalicylic acid (ASA) and high-dose intravenous immunoglobulin (IVIG) [once (67%), or twice (20%)], whereas a minority of cases (13%) was initially missed and did not receive any treatment. The difference in median age for cCTA when compared to CMR [16.5 years (1–59) vs. 12 years (0–57)], medians, and ranges), is in part explained by the earlier availability of the non-invasive CMR modality whereas the third generation dual-source cCTA only became available in 2015. Anesthesia was used in two patients for CMR as well as for cCTA because of their young age. The remaining patients were scanned while conscious and alert, medication to manage heart rhythm (i.e., beta-blockers) were not routinely used, only when the heart rate exceeded 70–75 beats per minute. The two patients with a history of coronary artery bypass grafting (CABG) were excluded from analysis.

## CAA Detection

With respect to the accuracy of coronary abnormalities we identified a total of 30 CAAs in 14 patients upon cCTA against 15 CAAs upon CMR in eight patients (**Table 2**). When the CAAs, visualized by cCTA were considered valid and true, the

**TABLE 2 |** Total CAAs detected by cCTA vs CMR.

| Coronary artery | CAA on cCTA | CAA on CMR |
|---|---|---|
| LMCA | 7 | 3 |
| RCA | 12 | 7 |
| LAD | 10 | 3 |
| Cx | 1 | 0 |

**TABLE 3 |** CAAs missed by CMR, with accompanying *Z*-scores calculated from the luminal diameters acquired by cCTA.

| LMCA in mm (*Z* score) | RCA in mm (*Z*-score) | LAD in mm (*Z*-score) | Cx in mm |
|---|---|---|---|
| 5 (1.25)[a] | 4 (3.3) | 4 (3.0) | 7 |
| 6 (4.26) | 7.4 (9.15) | 4 (2.52)[b] | |
| | 6 (3.88) | 6.4 (7.69) | |
| | | 9 (29.92) | |
| | | 7 (6.52) | |
| | | 5 (3.07) | |

[a] *Patient was overweight (BMI 35.1) which strongly affected the calculation of his Z-score.*
[b] *Borderline Z-score of 2.52, but with an irregular wall and an internal diameter of >1.5 times that of the adjacent segment, hence counted as CAA.*

distribution of CAAs missed by CMR was as followed: four CAAs in the LMCA, three CAAs in the RCA, seven CAAs in the LAD, and one CAA in the Cx. There was no clear cut-off in diameter above which the CMR was able to detect the CAAs (**Table 3**), but predominantly determined by imaging quality overall instead.

In one patient, the CMR detected four CAAs in the RCA and one CAA in the Cx, while the cCTA detected only three CAAs in the RCA and a normal Cx. The delay between CMR and cCTA was 10 months. A month prior to the cCTA a CAG was performed, the results of the CAG were in concordance with the cCTA. Imaging in this patient was performed in the 1st year after onset of disease [also referred to as the dynamic phase (4, 11)] therefore, this discrepancy is most likely due to remodeling.

The CMR detected CAAs in three patients which actually had no CAAs in the coronary artery tree upon cCTA (neither in echocardiography) whereas the CMR detected a CAA in the LAD (1 patient) and in the Cx (2 patients). The delay between CMR and cCTA was 61, 67, and 59 months. However, imaging in these patients was performed long after onset of disease (>2 years), also referred to as the static phase (4), making the contribution of remodeling or normalization much less likely as the explanation for these discrepancies.

## cCTA and CMR; Logistics and Failure Rates

Diagnostic failure rates to accurately assess the presence of vascular lesions in these patients by either cCTA or CMR were 9.6% (a total of nine coronary arteries were failed to visualize in five patients) and 59.6% (a total of 108 coronary arteries were failed to visualize in 31 patients), respectively, when both modalities were compared with each other. The main reason that CMR data acquisition failed to accurately assess the presence of a vascular lesion and therefore 1 or more coronary arteries could not be interpreted, more often than in cCTA, was due to motion artifacts caused by (i.e.,) irregular breathing and insufficient image quality; all but two patients were subsequently assessed successfully by cCTA. The cCTA gave insufficient results in five cases (without anesthesia) because of motion artifact (4) and so-called "streaking" due to beam hardening and scatter (1). In three patients the coronary artery segments which were visualized insufficiently by cCTA could be assessed by CMR or echocardiography and were unaffected. In the other two patients the Cx was not visualized well-enough either by cCTA, CMR and echocardiography. These latter two patients had no history of CAAs in any of the other coronary branches though.

## CAA-Related Secondary Complications

Imaging by cCTA was able to detect additional vascular pathology in nine patients with coronary features such as calcification ($n = 8$), stenosis ($n = 4$), occlusion ($n = 1$) which were not observed by routine CMR. Coronary artery thrombosis ($n = 3$) was detected only once by CMR. In three patients CMR enabled us to identify myocardial infarction, cCTA revealed signs of myocardial infarction in two of these patients but with much less accuracy.

## Clinical Repercussions

As a consequence of the insufficient performance of CMR, of the eight patients diagnosed with CAA upon CMR, four patients had a second or third CAA that were identified by cCTA and not by CMR. This did not lead to a change of CAA classification, in other words, the missed CAA did not exceed the other visible CAA in *Z*-score and therefore did not have clinical repercussions. CMR missed CAAs in three additional patients because of diagnostic failure (mainly due to motion artifact as mentioned before) which led to subsequent imaging by cCTA. Of these, one patient needed to start acetylsalicylic acid based on the new results of the cCTA. Finally, in the last three patients redefinition by cCTA of initially missed CAAs led to a different classification, i.e., from "no CAA" to "small CAA" in two patients and near giant CAA (*Z*-score 9.15) in one patient (**Figure 1**, **Table 3**). This last patient needed to start acetylsalicylic acid and also underwent subsequent CMR cardiac stress testing to detect possible myocardial damage, as she was pregnant at the moment of redefinition by cCTA, which showed no myocardial ischemia.

# DISCUSSION

Our study in KD indicates that cCTA is the better modality to assess the coronary artery lesions in clinical practice when compared to CMR, and can be used for a more precise risk stratification and monitoring during follow-up of patients.

Third generation dual-source cCTA has proven to be of great value for the evaluation of the luminal diameter of the coronary artery compared to invasive coronary angiography in adults (12, 13) and in KD patients (14) as well as the detection of CAAs and CAA-related secondary complications in KD (4, 15–17). The strength of cCTA lies predominantly in visualizing the anatomy

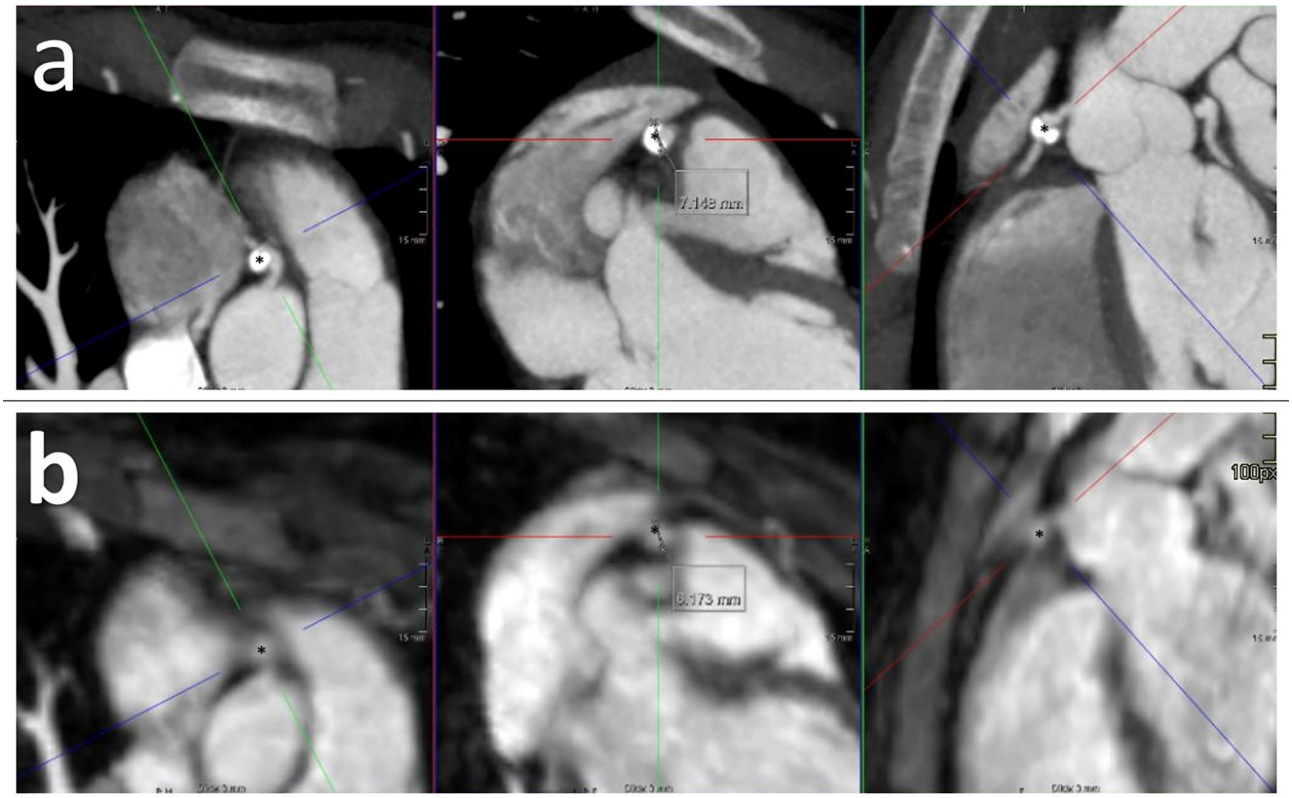


**FIGURE 1 |** cCTA imaging **(a)** and CMR imaging **(b)** of KD patient with near giant CAA (*) in RCA. Calcification and partial coronary artery thrombus are only visible upon cCTA.

and therefore to detect aneurysms, stenosis, calcification, and thrombosis. Other benefits of the third dual-source cCTA are the low radiation exposure and fast acquisition time leading to a lower burden on the pediatric population. Therefore, third generation dual-source cCTA appears to be a good candidate for coronary artery assessment in KD, i.e., risk stratification for the development of myocardial ischemia. However, CMR does not expose patients to radiation, unlike CT imaging and CAG. There is consensus that radiation exposure (due to imaging), is associated with an increase in lifetime cancer risk, especially in children and should be kept to a minimum (18, 19). Recent guidelines consider this radiation exposure and therefore mention CMR as a good imaging technique in KD (6), however, our data suggest cCTA is the more preferred modality for the visualization vascular morphology, thrombus formation and calcified lesions. CMR is valuable to visualize tissue characteristics and physiology, and has been used to demonstrate and in particular ischemia and tissue damage following myocardial infarction in KD (20, 21). Other imaging methods to evaluate cardiac function are being investigated as well (22). Current development in CT scanning techniques may enable cardiac function assessment in the future at low-dose radiation exposure (23, 24), but to date however, CMR remains the most suitable and best imaging modality for the evaluation of cardiac function and for detection of ischemia and infarction.

Differences in applicability and accuracy between CMR and cCTA have been described in the past in adults with coronary artery disease (CAD) (25). These patients mainly presented with obstructive CAD due to plaque formation and may not be comparable with our pediatric population with KD. Previous studies have attempted to determine the clinical applicability of either CT or CMR for the risk stratification (4, 12–17, 21, 26–29), but there are no comparative imaging studies available for KD to date.

Upon comparison of both modalities in daily practice, our data shows that cCTA outperforms CMR in the detection of CAAs. CMR showed a higher diagnostic failure rate for coronary artery assessment mainly due to motion artifacts caused by protracted acquisition time, compared to cCTA (respectively, 59.6 vs. 9.6%). To note, suboptimal images were rated as "diagnostic failure," as these suboptimal images led to the under-reporting of CAAs. The CAAs missed by CMR were more frequently localized in the LAD, followed by the LCA and RCA; of which three were missed because of inadequate performance.

Because echocardiography also had not been able to detect these CAAs, two patients went undertreated for 5 and 2 years, until their medium-sized CAAs were detected and oral medication was (re)started. In four patients, the CAA classification changed from normal to "small CAA," which needed no further medication. The four patients in whom

additional CAAs were detected that had been missed upon CMR, were already taking oral aspirin, but will be monitored by repeated cCTA during follow-up as a consequence. Important factors that contribute in the higher CAA detection rate by cCTA are high spatial resolution of the cCTA (0.6 mm/pixel vs. 0.6 × 0.6 × 1 mm/pixel in MRI), and high temporal resolution. This higher spatial resolution also contributes to the detection of additional coronary artery pathology (i.e., calcification, stenosis, and thrombosis), while CMR was inaccurate or unable to do so. These CAA-related secondary complications are relevant to be properly diagnosed for treatment considerations during follow-up. Another important factor for accurate coronary artery assessment is heart rate. A higher heart rate is a disadvantage for accurate coronary artery assessment. In our study, the average age of the children undergoing cCTA, and as a consequence their heart rate (since younger children on average have a higher heart rate than older children), was higher than in those that were imaged by CMR. Instead of the heart rate at start, longer acquisition time, and variability in heart rate upon CMR is probably the reason for a higher failure rate and lack of sufficient accuracy in CAA detection in KD. Stricter regulation of heart rate, managed by beta-blockers, could improve the diagnostic accuracy of CMR.

### Limitations

Retrospective analysis introduces variation of the data, having not been systematically collected in a predefined prospective manner. In our case, patients have been selected based on the prerequisite of having complementary imaging with both CMR and cCTA data available in the same patients. Most often these patients were known to have CAAs as previously visualized in the (sub)acute stage upon echocardiography. Thus, our patient cohort does not represent a normal unselected KD population. This, however, may not be a disadvantage because it is exactly this subgroup of patients that should be routinely monitored more closely. Despite the fact that most imaging was performed in the stable phase in which remodeling of coronary artery lesions is not expected anymore, the delay between both imaging techniques may have been of influence on our results. Since there were no luminal diameters available for some of the coronary arteries on CMR, the arteries were scored as "normal" or "abnormal" based on the experienced eye. This approach was considered the most realistic for subsequent comparisons between the two imaging groups (i.e., cCTA and CMR). The inability to measure the exact coronary artery diameter on CMR supports our first suspicion that CMR could have a lower diagnostic accuracy compared to cCTA.

Finally, we calculated the *Z*-scores using the McCrindle/Boston model, although not formally validated for being used for imaging by cCTA and CMR.

## CONCLUSION

Our study in KD shows that cCTA is an excellent imaging modality to assess the coronary artery tree at great resolution. cCTA detects CAAs more frequent and with greater detail when compared to CMR. Therefore, we recommend to perform cCTA in addition to echocardiography in CAA positive KD patients to detect and classify CAAs.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

DS conceptualized the study, collected data, and drafted the initial manuscript. IK and TK contributed equally as co-senior authors and conceptualized the study, coordinated, and supervised data collection and reviewed the manuscript for important intellectual content and revised the manuscript. NP conceptualized the study and reviewed for important intellectual content and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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# Kawasaki Disease Shock Syndrome in Japan and Comparison With Multisystem Inflammatory Syndrome in Children in European countries

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Multisystem inflammatory syndrome in children (MIS-C) is a severe Kawasaki-like illness that was first linked to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in European countries in the spring of 2020 and has been suggested to have overlap with Kawasaki disease shock syndrome (KDSS). There are few reports of MIS-C from Asia. This observational study aimed to identify the clinical features in children presenting with KDSS in Japan over a 5-year period and to summarize similarities and differences between KDSS and MIS-C. We retrospectively collected data on patient characteristics, clinical signs and symptoms, treatment, and prognosis including coronary artery abnormalities (CAAs), which were compared with data of patients with KDSS worldwide and patients with MIS-C from a review. KDSS was identified in 6 (1.1%) of 552 patients with Kawasaki disease (KD) treated at a single institution in Japan between 2015 and 2020 (1 in 2020). In patients with KDSS in Japan or worldwide vs. patients with MIS-C, KDSS was more likely to have a diagnosis of complete KD (100, 70 vs. 6.3%), a higher incidence of CAAs (50, 65 vs. 11%), and a greater requirement for vasoactive agonists (67, 67 vs. 43%) because of circulatory shock (100, 50 vs. 26%). Both KDSS and MIS-C had good prognosis (mortality 0, 6.7 vs. 1.7%). Although KDSS in Japan and MIS-C show some overlap in clinical symptoms, they are unlikely to be the same disease entity. KDSS is more likely to have a cardiovascular phenotype with CAAs and requires treatment with cardiovascular agents.

**Keywords: kawasaki disease, SARS-CoV-2, coronary artery, multi inflammatory syndrome-children, shock**

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## INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has had a major impact on pediatric medical care worldwide. In COVID-19, pneumonia is often mild in children, but when children develop multisystem inflammatory syndrome in children (MIS-C), also known as pediatric inflammatory multisystem syndrome temporally associated with severe acute respiratory syndrome coronavirus 2 (PIMS-TS), intensive care is required (1–3). As of spring 2020, this disease has been described in Western countries as a severe Kawasaki-like illness (4–8). Kabeerdoss et al. (9) have shown that MIS-C is a hyper-inflammatory state and can progress to MAS/cytokine storm syndrome.

Kawasaki disease is an acute vasculitis that occurs in childhood, and although its etiology is still unknown, it is known that many inflammatory cytokines are elevated. There have been many reports comparing KD and MIS-C, often stating that the two are partly similar but partly different (9–11). In particular, the overlap with KD shock syndrome (KDSS), which is KD complicated with circulatory failure and/or shock, is being discussed (12, 13). Few comparisons have been made between KDSS and MIS-C (9–13).

Currently, there are few reports on MIS-C from Asia (14, 15). The incidence of KDSS had been lower in Asia than in the US, Mexico, and European countries (16–22). There are very few case reports from Japan, which has the highest incidence of KD in the world (23). The aims of this study were to describe the clinical features and disease course in Japanese patients who presented with KDSS over a 5-year period and to discuss the similarities and differences between KDSS and MIS-C.

## PATIENTS AND METHODS

This retrospective observational study was conducted at a single center in Japan. Data on patient characteristics, clinical signs and symptoms, laboratory findings, treatment, and prognosis including coronary artery abnormalities (CAAs) were collected and compared with data of patients with KDSS worldwide (19) and patients with MIS-C in a review (24).

KDSS is defined in 2009 on the basis of an age-related decrease in systolic blood pressure, a sustained decrease of 20% or more in systolic blood pressure from baseline, or clinical signs of poor blood flow (12). Our 6 cases were diagnosed based on this definition.

Patient characteristics included age, sex, and date of starting treatment. Information on major symptoms of Kawasaki disease (KD) and cardiovascular, gastrointestinal, neural, and renal symptoms were collected. The results of laboratory investigations for each patient comprised blood cell counts including neutrophils and lymphocytes, coagulation profile, inflammation-related data including the erythrocyte sedimentation rate, and ferritin, and general biochemistry including hepatic and renal function, electrolytes, and brain natriuretic peptide (BNP) or N-terminal prohormone of brain natriuretic peptide (NT-pro BNP). We also examined the Kobayashi risk score, which predicts the likelihood of the illness being refractory to intravenous immunoglobulin (IVIG) therapy (25). We also collected data on treatment and outcomes, including CAAs.

We compared the data of KDSS patients in Japan with KDSS patients worldwide (19). Then, we compared these patients with patients with MIS-C from a review (24). Laboratory data were compared between our patients and patients with MIS-C in four previous papers (8–11). The raw data available for 10 patients with MIS-C in Italy and 6 cases of KDSS in Japan were statistically analyzed using the non-parametric Wilcoxon test. Categorical data on 655 cases of MIS-C were compared with data on the 106 cases of KDSS using Fisher's exact test (19, 24).

This study was approved by the Tokyo Women's Medical University Ethics Committee (#3636).

## RESULTS

KDSS was identified in 6 (1.1%) of 552 patients with Kawasaki disease (KD) treated at our institution in Japan between 2015 and 2020 (2 in 2015, 2 in 2018, 1 in 2019, and 1 in June 2020). The incidence of KDSS was 1.1% or 0.09 per month (**Figure 1**). There was no increase in the number of patients with KD or KDSS in 2020.

### Patient Characteristics

The median age of the 6 patients with KDSS was 3.5 years in Japan, which was younger than that of 106 patients with KDSS worldwide (19). There were 655 patients with MIS-C and they were older than the KDSS patients (**Table 1**). The male to female ratio was 1:1 in our small cohort but 1.2:1 in the KDSS patients worldwide and MIS-C patients.

### Clinical Features

In our 6 KDSS cases, the median day of illness at the first visit was day 6 (range, 4–6). Three patients had more than five primary symptoms at the first visit and the other 3 patients had six symptoms at the start of treatment (**Table 2**). Diagnosis of KD was straightforward in all cases. Shock was eventually diagnosed in the 6 patients. The onset of shock was before treatment in 3 cases (Cases 4–6 in **Table 2**), at 5 and 8 h after the first dose of IVIG in 2 cases (Cases 1 and 3), and immediately after the start of the second dose of IVIG in 1 case (Case 2). Five (88%) of the 6 patients reported gastrointestinal symptoms, which were found to be accompanied by gallbladder enlargement on abdominal echography. One patient had acute kidney injury. The median Kobayashi risk score at admission was 6 (range, 2–9).

In KDSS patients worldwide, 72 of 106 (70%) had complete KD. In MIS-C, the frequency of a diagnosis of complete KD was 6.3% (**Table 1**). The frequency of patients who met KD criteria in MIS-C was significantly lower than that in KDSS ($p < 0.01$) (24).

Gastrointestinal symptoms were common in both KDSS and MIS-C (**Table 1**). Cardiovascular symptoms were present in all patients with KDSS in Japan but in only about 50% of those with KDSS worldwide or MIS-C (19, 24).

### Laboratory Findings

Notable features of the KDSS were a high neutrophil ratio, a platelet count of $\leq 30 \times 10^4/\mu L$, and a high bilirubin level (**Table 2**). These features have previously been reported in high-risk patients with KD (25–27).

We obtained laboratory data of patients with MIS-C from four previous papers in Europe and the US (5–8). The increase in the neutrophil ratio and the decreases in lymphocyte count and hemoglobin level were similar in KDSS and MIS-C. However, the decrease in platelets was more pronounced in MIS-C (**Table 3**). The difference was significant when we compared data in our 6 patients to those in 10 patients in Italy ($p = 0.0011$) (5). With regard to inflammation markers, the C-reactive protein, ferritin levels, and the erythrocyte sedimentation rate were similar in both KDSS and MIS-C. No data were reported for bilirubin in MIS-C patients. The D-dimer value as a coagulation parameter could not be evaluated due to lack of data in KDSS patients.

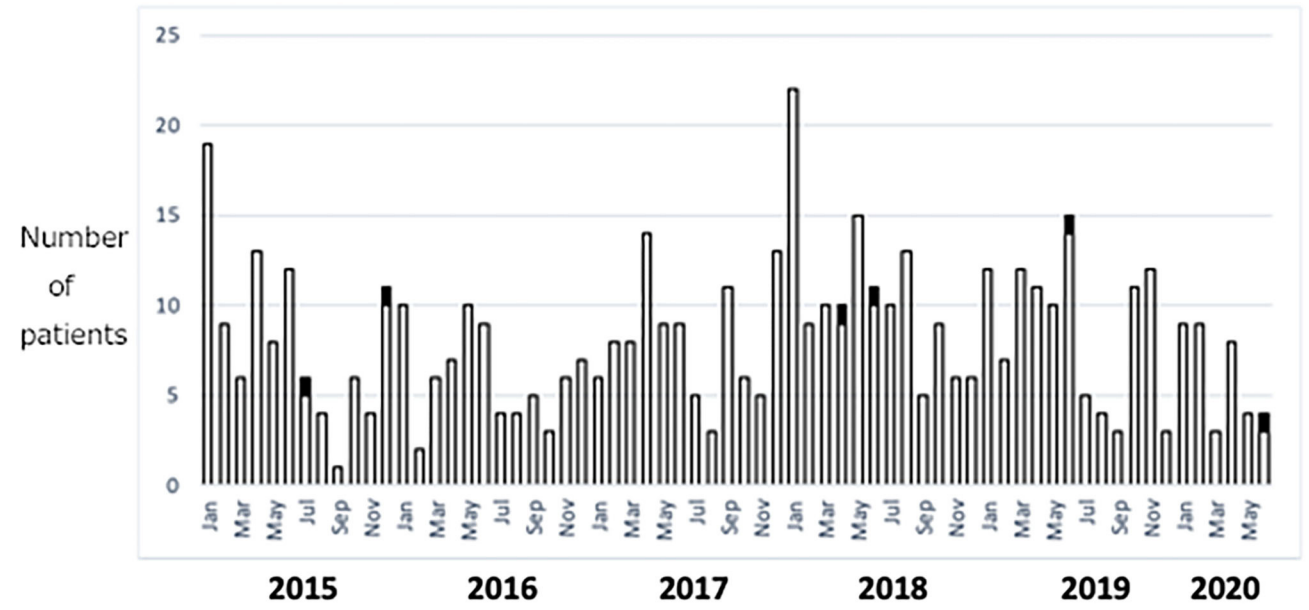


**FIGURE 1 |** Cases of KD and KDSS between 2015 and 2020 at a single institution in Japan. White columns represent KD and black columns represent KDSS. KD, Kawasaki disease; KDSS, Kawasaki disease shock syndrome.

**TABLE 1 |** Comparison between patients with KDSS and those with MIS-C.

| Type of presentation | KDSS in this study ($n = 6$) | KDSS worldwide ($n = 106$) (19) | MIS-C ($n = 655$) (24) |
|---|---|---|---|
| Age at onset | 3.5 (3–12 y) | 5.0 y | 8 (3 mo−20 y) |
| Sex | | | |
| Male (%) | 50% | 55% | 55% |
| Hospital stay, d | 6 (4–6) | mean 7.2 | 4 (3–6) |
| Clinical Symptoms | | | |
| Fever | 100% | 100% | 100% |
| Gastrointestinal | 87% | 75% | 70% |
| Cardiovascular | 100% | 50% | 51% |
| Neurologic | 0% | 54% | 22% |
| Skin rash | 100% | ND | 58% |
| Respiratory | 0% | 32% | 9.6% |
| Renal | 13% | 46% | ND |
| Met KD criteria | 100% | 70% | 6.2% |
| Treatment and outcome | | | |
| Inotropes | 67% | 67% | 45% |
| Invasive mechanical ventilation | 0% | 28% | 20% |
| Death | 0% | 6.7% | 1.7% |

## Physical and Cardiovascular Function Associated With Shock

The 6 patients with KDSS had a median systolic blood pressure on admission of 78 (range, 72–93) mmHg, which is normal for children, but had minimum systolic blood pressures that were 30–39% lower than those recorded after recovery. However, the median diastolic blood pressure at admission was 41 (29–51) mmHg, which was within the normal range. The limbs were warm in all patients, indicating warm shock. On echocardiography, 3 of the 6 cases (50%) had a low cardiac ejection fraction during shock. Four patients (67%) had mild pericardial effusion. Second-degree atrioventricular block was observed in Case 3, which recovered by 1 month after onset of KD (**Table 2**).

Shock developed in all patients with KDSS in Japan but in 50% of patients with KDSS worldwide and in only 23% of patients with MIS-C (**Table 4**). BNP and NT-pro BNP levels were elevated in both KDSS and MIS-C (**Table 3**). Troponin data were available for only 2 patients with KDSS in Japan, precluding comparisons.

## Treatment

In the 6 patients with KDSS, treatment was started on median day 6 of illness. All patients received IVIG plus aspirin. Cyclosporine A was administered with IVIG and aspirin in Case 6 (**Table 2**). Three of the 6 patients were resistant to the initial dose of IVIG and required up to third-line treatment for KD. Four of the 6 patients received inotropic agents, including dobutamine and a phosphodiesterase (PDE) inhibitor. The response to these agents was very good in all cases. The first patient received dobutamine 3 μg/kg/h, which was tapered over 4 days (Case 1 in **Table 2**). The second patient received a combination of dobutamine and a PDE inhibitor (Case 2); the initial dose of dobutamine was 3γ and that of the PDE inhibitor was 0.1γ, with a treatment duration of 4 days. The third patient received dobutamine over a period of 26 h (Case 3). A combination of dobutamine and

**TABLE 2 |** Clinical and laboratory features of 6 patients with KDSS.

| | Case1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 |
|---|---|---|---|---|---|---|
| Date of onset | June 2015 | Dec 2015 | Apr 2018 | June 2018 | June 2019 | June 2020 |
| Age, y | 12 | 4 | 6 | 3 | 3 | 3 |
| Sex | M | F | M | F | F | M |
| Day of illness at KD diagnosis | 6 | 4 | 6 | 6 | 6 | 5 |
| Day of illness at shock | 6* | 9* | 7* | 6 | 6 | 5 |
| Type of KD | Complete | Complete | Complete | Complete | Complete | Complete |
| Conjunctival injection | Yes | No | Yes | Yes | Yes | Yes |
| Lips and tongue | Yes | Yes | Yes | Yes | Yes | Yes |
| Rash | Yes | Yes | No | Yes | Yes | Yes |
| Swollen hands and feet | Yes | Yes | Yes | Yes | Yes | Yes |
| Lymphadenopathy | Yes | Yes | Yes | Yes | Yes | Yes |
| Other symptom | | | | | | |
| Abdominal pain | Yes | Yes | No | Yes | No | No |
| Diarrhea | No | Yes | No | No | No | No |
| Vomiting | No | No | No | No | No | Yes |
| Shock | Yes | Yes | Yes | Yes | Yes | Yes |
| Renal failure | No | Yes | No | No | No | No |
| Others | | | A-V block | | | |
| Bacterial culture and antigen exam | GAS+ | No | No | No | No | COVID-19 PCR (–) |
| CRP (mg/dL) | 23 | 17.2 | 26.7 | 28.5 | 10.3 | 5.07 |
| ESR (mm/h) | 93 | 68 | 115 | 123 | 122 | ND |
| White blood cell count | 9.14 | 25.97 | 13.5 | 21.18 | 13.7 | 12.5 |
| Neutrophils (%) | 93 | 89 | 85 | 92 | 86 | 83 |
| Lymphocytes (%) | 2.7 | 8.9 | 12.1 | 4.6 | 9.1 | 14 |
| Hemoglobin (g/dL) | 12.6 | 8.6 | 10.2 | 9.2 | 10.9 | 13.4 |
| Platelets ($\times 10^3$) | 23.1 | 31.9 | 25.1 | 19.6 | 21.4 | 26.6 |
| Albumin (g/dL) | 2.7 | 2.0 | 2.0 | 1.7 | 2.8 | 3.5 |
| Sodium (mEq/L) | 134 | 128 | 129 | 135 | 129 | 136 |
| AST (U/L) | 50 | 19 | 44 | 33 | 44 | 39 |
| ALT (U/L) | 85 | 21 | 77 | 25 | 31 | 77 |
| Total bilirubin | 5.5 | 4.2 | 1.2 | 1.2 | 0.4 | 0.4 |
| Ferritin (ng/mL) | 713.8 | 548 | ND | 447 | 646 | 175.9 |
| D-dimer | 22.92 | ND | ND | ND | ND | ND |
| CPK | 14 | 235 | 52 | 11 | 22 | 36 |
| Troponin I | 0.03 | ND | ND | ND | ND | 15.8 |
| BNP (pg/mL) | 96.6 | 678 | 1179 | 1764 | 789.8 | 568 |
| Kobayashi score | 6 | 9 | 6 | 2 | 6 | 5 |
| Pneumonia on chest X-ray | No | No | No | No | No | No |
| Ejection fraction before treatment | 68 | 69 | 69 | 38 | 44 | 48 |
| Mitral valve regurgitation | No | Yes | Yes | No | No | Yes |
| Pericardial effusion | Yes | Yes | Yes | Yes | No | No |
| Coronary artery lesion | Yes | Yes | Yes | No | No | No |
| Inotropes | Yes | Yes | Yes | Yes | No | No |
| Response to treatment | Yes | Yes | Yes | Yes | Yes | Yes |

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BNP, brain natriuretic peptide; CPK, creatine phosphokinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; KD, Kawasaki disease; ND, no data; *During/after IVIG treatment.*

a PDE inhibitor was administered in the fourth patient; the respective initial doses were 2 and 0.05γ and the treatment duration was 4 days (Case 4). No inotropic agent was used in Case 5 and Case 6. Systolic blood pressure during use of inotropic agents was over 110 mmHg in 3 of the 4 patients who received inotropic support.

In the KDSS patients worldwide, 67% were treated with inotropic agents. In the MIS-C patients, 45% were treated with

**TABLE 3 |** Laboratory data.

| Type of presentation | KDSS in this study ($n = 6$) | KDSS worldwide (19) ($n = 106$) | MIS-C in Italy (5) ($n = 10$) | MIS-C in UK (6) ($n = 58$) | MIS-C in NewYork (7) ($n = 99$) | MIS-C in US (8) ($n = 186$) |
|---|---|---|---|---|---|---|
| CRP (mg/dL) | 20.1 (12.0, 25.8) | 19.1* | 24.1 (9.7, 27.9) | 22.9 (156, 338) | 21.9 (15.0, 30.0) | 17.8 (12.8, 25.9) |
| ESR (/mm) | 115 (93, 122) | 61.9* | 75.5 (51, 97) | ND | 61.5 (43.0, 77.5) | 65 (42, 91) |
| White blood cell count ($\times 10^3/mm^3$) | 13.6 (12.8, 19.3) | 17.3* | 10.8 (6.1)* | 17 (12, 22) | 10.4 (6.7, 14.5) | ND |
| Neutrophils (%) | 87.5% (85.3, 91.3) | 81.0* | 84.8% (78.6, 90.3) | 13 (10, 19) | 82.0% (76.0–89.0) | ND |
| Lymphocytes ($\times 10^3/mm^3$) | 1.44 (1.04, 1.72) | ND | 0.83 (0.46, 1.12) | 0.80 (0.50, 1.50) | ND | ND |
| | 9.0% (5.7, 11.4) | | | | 10.0% (5.0, 16.0) | |
| Hemoglobin (g/dL) | 10.6 (9.2, 12.6) | 11.1* | 11 (1.2)* | 9.2 (8.3, 10.3) | ND | <9.0: 48% |
| Platelets ($\times 10^4/mm^3$) | 24.1 (21.4, 26.6) | ND | 13.0 (11.6, 14.2) | 15.1 (10.4, 21.0) | 15.5 (10.5, 23.3) | 13.3 (8.8, 23.5) |
| ALT (IU/L) | 41.5 (25, 77) | 97.1* | 56 (26, 79) | 42 (26, 95) | ND | ND |
| Albumin (g/dL) | 2.4 (2.0, 2.8) | 2.4* | 3.2 (0.3)* | 2.4 (2.1, 2.7) | 3.1 (2.5, 3.6) | 2.5 (2.0, 2.9) |
| Ferritin (ng/mL) | 548 (447, 646) | ND | 893 (324, 2000) | 610 (359, 1280) | ND | 639 (332.7, 1178.2) |
| CPK (U/L) | 29 (22, 52) | ND | 78 (40, 87) | ND | ND | ND |
| Elevated BNP or NT-pro BNP (%) | 100% | ND | 100% | ND | 90% | ND |

*Median (IQR). *Mean. ND, No data.*

**TABLE 4 |** Cardiovascular phenotype in KDSS and MIS-C.

| Type of presentation | KDSS in this study ($n = 6$) | KDSS worldwide ($n = 91$) (19)* | MIS-C ($n = 655$) (24) |
|---|---|---|---|
| Ejection fraction ≤55 | 50% | 45%** | 32% |
| Myocarditis | 100% | ND | 23% |
| Mitral valvular regurgitation | 50% | 14% | ND |
| Coronary artery abnormalities | 50% | 65% | 11%† |
| Circulatory shock | 100% | 50% | 26% |

*Patients in whom echocardiography was obtained. **Ejection fraction <50%.
†Among 482 patients in whom echocardiography was obtained. ND, No data.*

inotropic agents, which is a smaller proportion than in the patients with KDSS in Japan (**Table 1**).

## Coronary Artery Abnormalities and Mortality

Three of the 6 patients with KDSS in Japan (50%) had CAAs. Case 1 had a giant aneurysm, and Cases 2 and 3 had transient dilation. This high incidence of CAAs was similar in KDSS patients worldwide (**Table 4**) (19). Patients with MIS-C had CAAs at a frequency of 11% among 482 patients in whom echocardiography was obtained (24).

There were no deaths from KDSS in Japan, whereas 6.7% of patients with KDSS worldwide and 1.7% of patients with MIS-C died. The US Centers for Disease Control and Prevention reported 20 deaths among 1,027 cases of MIS-C (2%) as of October 12, 2020 (28).

# DISCUSSION

There are few reports of KDSS in Japan (23), and there is insufficient information on the differences in clinical symptoms between cases in Japan and cases in Europe and the US. We have encountered 6 cases in 5 years based on the definition of Kanegaye et al. (12). The frequency in Japan is 1.1%, which is less than the rate of 5–7% reported in Europe and the US (16–18). The frequency is also 1–3% in Taiwan and China, and there are reports suggesting that KDSS is less common in Asians (20–23). The features of KDSS identified in this study, such as patients being older than those with KD without shock, presence of more abdominal symptoms, meeting the diagnostic criteria for KD, likelihood of being refractory to IVIG, and use of vasoactive agents in two-thirds of cases, are consistent with the features reported in a review of KDSS centered on Europe and the US (19). However, in the review, the frequency of requirement for mechanical ventilation (28%) and the mortality rate (6.8%; 7 patients, 3 of whom had myocardial infarction) were different from our experience. Access to medical care is good in Japan, and diagnosis of KD is rapid given the relatively high frequency of the disease in Japan. On average, treatment is started on day 4 of illness. Early treatment may help to reduce mortality.

KD, and especially KDSS, has received considerable attention due to the outbreak of MIS-C in 2020. Two to 4 weeks after the onset of SARS-CoV-2 infection, inflammation spreads to multiple organs. Many children present with disorders affecting the circulatory, gastrointestinal, renal, and other organ systems, and this condition is diagnosed as MIS-C or PIMS-TS (1–3). MIS-C was reported to have clinical symptoms similar to those of KDSS (4–9). Many more cases of KDSS have been registered since March 2020 than were diagnosed until 2019 (4–8). However, there are very few reports of MIS-C in Asia, including Wuhan, which is the birthplace of SARS-CoV-2 (14, 15). As shown here, the number of cases of KDSS did not increase in 2020 in Japan. In 2020, we had 1 case of KDSS, which was negative for SARS-CoV-2 by polymerase chain reaction (antibodies untested). The total number of KD cases in Japan is lower in 2020 than in 2019.

Patients with KDSS are older than those with KD without shock, but the percentage under 5 years of age was reported to be 56% in the review of KDSS (19) and was 67% in our case series. In contrast, about 25% of patients with MIS-C were over 12 years old. It has been reported that 55% of patients with KDSS worldwide are boys (19) and that the sex ratio for MIS-C is the same (24).

The pathophysiology of KDSS is understood to be mainly arteritis and myocarditis with lesions in multiple organs. In our experience, the limbs are warm and KDSS is considered to be a mixed type of shock due to decreased cardiac contraction and hyperpermeability of the peripheral arteries (18). In MIS-C, whether or not the pathophysiology of shock is similar to that of KDSS is an important question.

The rate of CAAs is much higher in KDSS than in MIS-C. The frequency in KDSS was significantly higher than that in the MIS-C ($p < 0.01$) (19, 24). Some patients with MIS-C present with coronary aneurysm similar to KD; however, the definition of MIS-C differs slightly between the US, Europe, and the World Health Organization, and since it is not strict, some KD cases could be mixed in with MIS-C cases. SARS-CoV-2 infection promotes release of an angiotensin-converting enzyme receptor in the vascular endothelium and has been reported to cause vasculitis (29), but we would like to know whether the main focus of inflammation is arteries or veins. Recently, Consigio et al. (30) published a comprehensive analysis of peripheral blood immune cells and blood cytokines in patients with KD and MIS-C and reported that the inflammatory response in MIS-C differs from that in KD with respect to T cell subsets, some cytokines, and biomarkers associated with arterial damage.

Gastrointestinal symptoms are also common in KDSS, particularly vomiting and abdominal pain, which are caused by cholestasis (19). We believe these symptoms may also be caused by gastrointestinal hypoperfusion due to heart failure. In MIS-C, the main symptom is reported to be diarrhea (4–8). We would like to know about gallbladder swelling and the blood bilirubin level in MIS-C. Renal failure, nervous system disorders, and respiratory symptoms were reported to occur with similar frequency in KDSS and the MIS-C (5–8, 19, 24).

In our study, the ferritin level, which is important for the diagnosis of MIS-C, was not markedly different between patients with MIS-C and those with KDSS. However, serum ferritin levels were extremely high in some patients with MIS-C, and we consider that further investigation is needed before we can draw a conclusion. Platelet counts were often below 300,000/μL in both diseases but were lower in patients with MIS-C. Although measurements of D-dimer and fibrinogen were insufficient in this study, we think that the pathophysiology of MIS-C is closer to that of macrophage-activating syndrome.

Both diseases had much in common in terms of treatment, with management in the intensive care unit in 70–80% of cases and ventilation management in 20–30%; about 50% were refractory to IVIG, and steroids were used in 35%. Use of vasoactive agents was significantly higher in the KDSS patients than in the MIS-C patients (67 vs. 43%; **Table 1**), which reflects the fact that the phenotype of KDSS includes more severe heart failure. The mortality rate for the two diseases is similar at 0–6.7% for KDSS and 0–2% for MIS-C (4–8, 28) (**Table 1**).

This study has several limitations. First, the number of KDSS cases was small, and some of our findings were different from those reported previously for KDSS, so we did not adequately perform statistical analysis. Therefore, we cannot reach any definitive conclusion based on our results. Second, the diagnostic criteria for both KDSS and MIS-C are symptom-based diagnoses, and their clinical symptoms overlap. Of course, there may be patients who meet both diagnostic criteria for both diseases. Third, the study had a retrospective design, which meant that only a limited number of features could be compared between KDSS and MIS-C.

## CONCLUSION

KDSS in Japan and MIS-C in European countries have some similar clinical signs and symptoms, but despite this overlap are unlikely to be the same disease entity, in light of the diagnostic criteria for KD and the incidence of CAAs, which are features of the most important phenotype of KD. KDSS has a predominantly cardiovascular phenotype and requires more treatment with circulatory agents. Analysis of the pathophysiology of MIS-C has started, and further studies focusing on vasculitis are awaited to elucidate the size of blood vessels and whether arteries or veins are mainly involved.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Tokyo Women's Medical University Ethical Committee. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

JS analyzed the data and wrote the manuscript. KA analyzed the data. TM, KY, and TH critically reviewed the manuscript. J-iT reviewed the research and monitored ethical issues. HH designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

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# Kawasaki Disease and Systemic Juvenile Idiopathic Arthritis – Two Ends of the Same Spectrum

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Kawasaki disease (KD) and systemic juvenile idiopathic arthritis (sJIA) are two distinct systemic inflammatory diseases of childhood. Each diagnosis is based on criteria, but numerous clinical features are overlapping. As no specific diagnostic tests are available, differentiation between both disease entities can be challenging. Here, we describe the disease course of patients with co-diagnosis of both KD and sJIA (KD/sJIA). All our KD ($n = 1765$) and sJIA ($n = 112$) cases were critically reviewed for co-diagnosis of KD/sJIA. Eight KD/sJIA cases were identified and their clinical presentation, treatment regimens, coronary artery outcome and complications are herein described. Each KD/sJIA patient fulfilled diagnostic criteria for KD and for sJIA. Ongoing fever, rash and arthritis were present in each patient. The KD/sJIA patients had recalcitrant KD requiring multiple doses of intravenous immunoglobulin and steroids. Five patients had coronary artery dilatation at KD diagnosis, which resolved in all by 6 weeks. Pericardial effusion was present in 5 patients. One KD/sJIA patient developed macrophage activation syndrome. In conclusion, a small proportion (0.5%) of our KD patients evolved into sJIA, and 7% of our sJIA population presented initially as KD. KD/sJIA patients were characterized by a recalcitrant KD course and a high prevalence of coronary artery dilatation. Patients with co-diagnoses may provide a clue to potentially shared immunopathology in KD and sJIA, leading us to posit that both entities may be part of the same clinical spectrum.

**Keywords: kawasaki disease, juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, macrophage activation syndrome, fever, interleukin 1**

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## INTRODUCTION

Kawasaki disease (KD) and systemic juvenile idiopathic arthritis (sJIA) are two distinct systemic inflammatory diseases of childhood. Each diagnosis is based on criteria, but numerous clinical features are overlapping. As no specific diagnostic tests are available, differentiation between KD and sJIA can be challenging. Recent research suggests underlying biologic similarities between these two childhood inflammatory diseases with involvement of interleukin-1 beta (IL-1β). We critically reviewed patients with combined diagnoses of KD and sJIA (KD/sJIA) in order to characterize discriminating findings at baseline and provide evidence for their potentially shared immunopathology.

## MATERIALS AND METHODS

Medical records of all patients diagnosed with either KD ($n = 1765$) or sJIA ($n = 112$) at a tertiary referral center between January 1990 and December 2011 were reviewed. KD/sJIA patients ($n = 8$) were included who both fulfilled the American Heart Association guidelines for KD (1) and the International League of Associations for Rheumatology (ILAR) classification criteria for sJIA (2). Data from the records included demographic information, clinical features, laboratory results, echocardiographic findings, medical treatment and outcome.

Data were summarized as frequencies with percentages, means with standard deviation or median with interquartile range. Data were analyzed using *t*-test and a *P*-value of $<0.05$ was considered significant.

## RESULTS

### Patient Characteristics and Treatment

In **Table 1**, we compared baseline characteristics, treatment received and disease complication between KD and KD/sJIA patients. In the KD cohort, median age at diagnosis is 3.1 years (IQR 1.7-5.3), majority of whom were between age range of 1-9 years old (81%) and male (62%). The KD/sJIA patients were diagnosed with KD at median age of 4.7 (IQR 2.1-5.6), mostly male (75%) and no one presented with symptoms at <1 year or >9 years of age. Conjunctival injection was less frequent noted among all the KD features. All of them had rash and half had incomplete KD features, although not statistically significant.

KD patients had higher alanine transaminase (median of 28, IQR 16–67 U/L, vs. 21, IQR 11–29 U/L) and aspartate transaminase (median of 37, IQR 27–55 U/L, vs. 30, IQR 28–52 U/L), while KD/sJIA patients had lower albumin (mean of 29 ± 5 vs. 36 ± 6 g/L) and higher white blood cell count (median of 17.0, IQR 15.1–20.6 × $10^9$/L, vs. 13.0, IQR 9.3–16.9 × $10^9$/L) at diagnosis.

A significantly greater proportion of KD/sJIA patients received more than one dose of intravenous immunoglobulin (IVIG) and steroids.

### KD/sJIA Case Summary

Detailed clinical features of all patients are reported in **Table 2** and **Figure 1** illustrates a typical disease course of a KD-sJIA patient.

**Patient 1** is a 23-month-old male who presented with 6 days of fever and 3/5 KD diagnostic criteria with coronary artery (CA) dilatation seen on echocardiography. He was treated with IVIG twice and high dose acetylsalicylic acid (ASA), but remained febrile. He improved after high dose intravenous methylprednisolone pulses (IVMP) and was discharged with a tapering dose of prednisone. The CA dilatation resolved by the 6 week echocardiography follow-up. Two weeks later he developed spiking fevers and arthritis leading to suspicion of sJIA. Recurrent fever and highly elevated inflammatory markers persisted and ultimately he responded well to Anakinra (IL-1 receptor antagonist). Seventeen months post-diagnosis of sJIA, he was in clinical remission and off medication.

**TABLE 1 |** Baseline features at diagnosis, medication and complication*.

| | KD ($n = 1765$) | KD/sJIA ($n = 8$) | *P*-value† |
|---|---|---|---|
| **Demographics** | | | |
| Sex (male) | 1,091 (62%) | 6 (75%) | 0.72 |
| Age at diagnosis, years (median, IQR) | 3.1 (1.7–5.3) | 4.7 (2.1–5.3) | 0.81 |
| Less than 1 year old | 218 (12%) | 0 (0%) | 0.61 |
| Greater than 9 years old | 130 (7%) | 0 (0%) | 1.00 |
| **Classic Kawasaki disease clinical signs** | | | |
| Number of days of fever pre–diagnosis | 6 (5–8) | 5 (5–6) | 0.20 |
| Incomplete Kawasaki disease | 483 (29%) | 4 (50%) | 0.24 |
| Bilateral conjunctival injection | 1,433 (88%) | 4 (50%) | 0.01 |
| Oral changes | 1,424 (87%) | 7 (88%) | 1.00 |
| Extremity changes | 1,250 (77%) | 6 (75.0%) | 1.00 |
| Polymorphous skin rash | 1,436 (88%) | 8 (100%) | 0.61 |
| Cervical lymphadenopathy | 962 (59%) | 3 (38%) | 1.00 |
| **Laboratory investigations** | | | |
| Albumin (g/L) | 35 ± 6 | 29 ± 5 | 0.01 |
| Alanine transaminase (U/L) | 28 (16–67) | 21 (11–29) | < 0.001 |
| Aspartate transaminase (U/L) | 37 (27–55) | 30 (28–52) | 0.04 |
| C-reactive protein (mg/L) | 35 (28–47) | 35 (31–202) | 0.32 |
| Erythrocytes sedimentation rate (mm/h) | 68 (41–95) | 104 (62–120) | 0.08 |
| Hematocrit | 0.334 (0.311–0.358) | 0.322 (0.266–0.329) | 0.11 |
| Hemoglobin (g/L) | 113 ± 13 | 102 ± 17 | 0.13 |
| Lymphocytes ($10^9$ cells per L) | 2.6 (1.6–4.2) | 1.9 (1.0–3.6) | 0.09 |
| Platelets ($10^9$ cells per L) | 355 (267–462) | 408 (315–546) | 0.54 |
| Red blood cells ($10^{12}$ cells per L) | 4.2 ± 0.5 | 3.9 ± 0.4 | 0.14 |
| White blood cells ($10^9$ cells per L) | 13.0 (9.3–16.9) | 17.0 (15.1–20.6) | 0.03 |
| **Treatment** | | | |
| Multiple intravenous immunoglobulin | 221 (14%) | 6 (75%) | < 0.001 |
| Intravenous steroids | 115 (7%) | 7 (88%) | < 0.001 |
| Oral steroids | 56 (3%) | 5 (63%) | < 0.001 |
| **Complications** | | | |
| No coronary artery aneurysms (z-score <2.5) | 1,271 (86%) | 6 (75%) | 0.32 |
| Small coronary artery aneurysms (z-score 2.5–5.0) | 125 (9%) | 2 (25%) | 0.14 |
| Large coronary artery aneurysms (z-score 5.0–10.0) | 40 (3%) | 0 (0%) | < 0.001 |
| Giant coronary artery aneurysms (z-score >10) | 43 (3%) | 0 (0%) | < 0.001 |
| Macrophage activation syndrome | 16 (1%) | 1 (13%) | 0.07 |

**Data are reported as frequencies with percentages, means with standard deviation or median with interquartile range.*

*† Obtained from comparison between patients from the KD cohort and KD patients with a co-diagnosis of sJIA.*

*KD, Kawasaki disease; sJIA, systemic idiopathic arthritis; KD/sJIA, Kawasaki disease patients with a co-diagnosis of systemic juvenile idiopathic arthritis.*

**Patient 2** is a 23-month-old female who presented with 12 days of fever and 3/5 diagnostic criteria for KD plus arthritis and CA dilatation on echocardiography. Treatment of incomplete

**TABLE 2 |** Clinical characteristics of KD/sJIA patients.

| Clinical characteristic | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 |
|---|---|---|---|---|---|---|---|---|
| Sex | Male | Female | Male | Male | Female | Male | Male | Male |
| Age at KD diagnosis | 1 y, 11 mo | 1 y, 11 mo | 2 y, 3 mo | 4 y, 5 mo | 5 y, 0 mo | 5 y, 3 mo | 5 y, 6 mo | 7 y, 3 mo |
| **Kawasaki disease** | | | | | | | | |
| Days of fever at diagnosis | 6 | 12 | 5 | 5 | 12 | 6 | 5 | 5 |
| KD criteria fulfilled in addition to fever | 3/5 | 3/5 | 4/5 | 3/5 | 3/5 | 5/5 | 5/5 | 4/5 |
| Bilateral conjunctival injection | | | + | | + | + | + | |
| Oral changes | + | | + | + | + | + | + | + |
| Extremity changes | | | | | | | | |
| Acute | + | + | + | + | | | + | + |
| Subacute | | | | | | + | | |
| Polymorphous skin rash | + | + | + | + | + | + | + | + |
| Cervical lymphadenopathy | | + | | | | + | + | + |
| Treatment received for KD | | | | | | | | |
| Total doses of IVIG (2g/kg) | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 |
| Total methylprednisolone pulses | 3 | 4 | 1 | 3 | 3 | – | 3 | 3 |
| Oral steroids | + | + | + | + | | | | + |
| Coronary artery dilatation | + | + | | + | + | | | + |
| Complications | | MAS | | | | | | |
| **Systemic juvenile idiopathic arthritis** | | | | | | | | |
| Time between KD diagnosis and presumptive diagnosis of sJIA ◦ | 22 d | 8 d | 24 d | 30 d | 52 d | 23 d | 90 d | 18 d |
| Time between KD diagnosis and onset of arthritis | 24 d | 0 d | 24 d | 0 d | 52 d | 23 d | 2 d | 125 d |
| sJIA criteria fulfilled in addition to fever and arthritis | 2/4 | 4/4 | 4/4 | 3/4 | 3/4 | 3/4 | 2/4 | 3/4 |
| Evanescent rash | + | + | + | + | + | + | + | + |
| Generalized lymphadenopathy | + | + | + | | | + | + | + |
| Hepatomegaly and/or splenomegaly | | + | + | + | + | + | | |
| Serositis† | | + | + | + | + | | | + |
| Duration of fever after sJIA diagnosis, mo | 8 | 3 | 3 | 7 | 2 | 72 | 2.5 | 10 |
| Treatment received for sJIA | | | | | | | | |
| NSAIDs | + | + | + | + | + | + | + | + |
| Oral prednisone | + | + | + | + | | + | + | + |
| DMARDs | | | | | | + | | |
| Biologics | Anakinra | | Canakinumab | | | Etanercept | | |
| Other | | | | | | IVIG | | IVIG |
| Length of follow-up | 17 mo§ | 12 mo*‡ | 18 mo* | 10 y§ | 2 y*‡ | 13 y* | 11 y§ | 6 y§ |
| Disease course | monocyclic | undetermined | undetermined | monocyclic | polycyclic | chronic | monocyclic | chronic |

*KD/sJIA, co-diagnosis of Kawasaki disease and systemic juvenile idiopathic arthritis; KD, Kawasaki disease; IVIG, intravenous immunoglobulin; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease modifying anti-rheumatic drugs; y - year(s); MO, month(s); D, day(s).*

*+Indicates that the symptom was present or treatment was given; – indicates that treatment was not utilized.*

*◦Presumptive diagnosis of sJIA before the criterion of 6 weeks of arthritis was met.*

*†All patients had pericarditis and two patients had also pleural effusion.*

**Indicates patient was on medication at the last clinic visit.*

*§Indicates patient was off medication at the last clinic visit.*

*‡Indicates patient was lost to follow-up.*

KD consisted of two doses of IVIG and high dose ASA with no clinical improvement. She progressed to have polyarthritis, developed serositis (pleural and pericardial) and her disease evolved into biopsy-proven MAS. She was then diagnosed with sJIA. After high dose IVMP she defervesced and improved clinically, requiring oral steroids for ongoing rash and arthritis. The CA dilatation had resolved by the 6 weeks. One-year post-diagnosis of sJIA, her symptoms were well-controlled with a low dose of prednisone.

**Patient 3** is a 27-month-old male who presented with 5 days of fever and 4/5 KD criteria and pericardial effusion on echocardiography. He received IVIG twice and high dose ASA but remained febrile and was given high dose IVMP followed by oral prednisone with good response and resolution of symptoms.

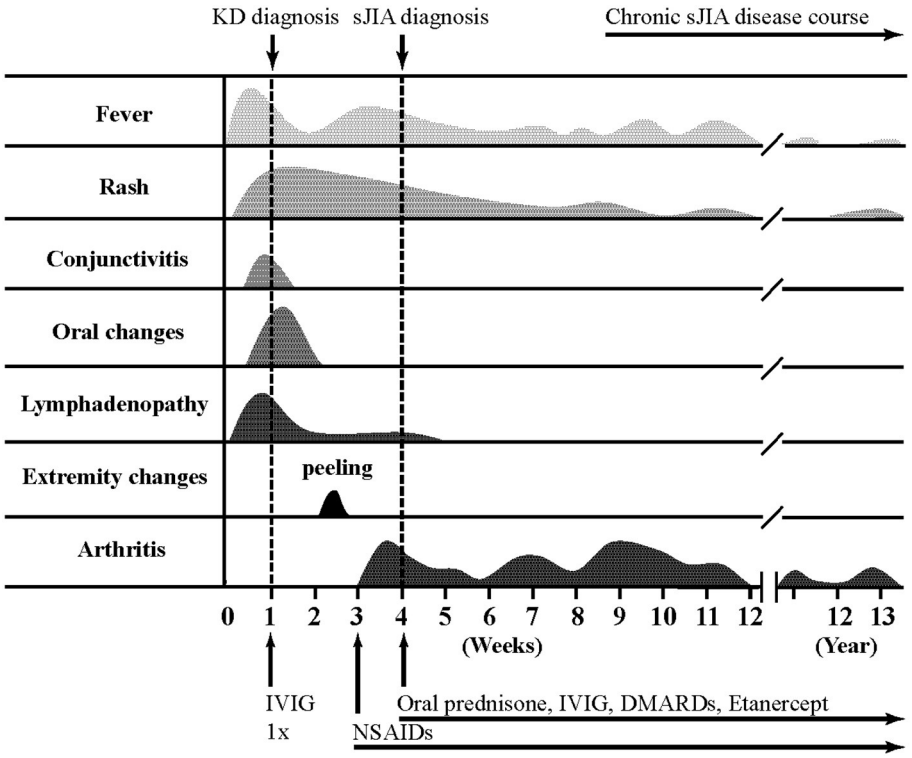


**FIGURE 1 |** Typical disease course of Kawasaki disease-systemic juvenile idiopathic arthritis.

Several weeks after the KD diagnosis, fever recurred with rash and arthritis and consequently he was diagnosed with sJIA. Canakinumab (anti IL-1β) was started for ongoing inflammation. Eighteen months post-diagnosis of sJIA he was in clinical remission on medication.

**Patient 4** is a 4-year-old male who presented with 5 days of fever and 3/5 KD criteria together with clinical signs of congestive heart failure and arthritis. He was treated with high dose ASA, IVIG once and IVMP with resolution of his inflammation. His echocardiography showed pericardial effusion, myocardial dysfunction and CA dilatation. A month after KD diagnosis he again developed rash and arthritis and was diagnosed with sJIA. The CA dilatation had resolved at 6 weeks echocardiography follow-up. Ten years post-diagnosis of sJIA, he was in clinical remission and off medications.

**Patient 5** is a 5-year-old female who presented with 12 days of fever and 3/5 KD features with CA dilatation and pericardial effusion on echocardiography. She was treated with IVIG twice and high dose ASA with minimal response, but improved after high dose IVMP. The CA dilatation had resolved at 6 weeks. Two months later she was diagnosed with sJIA due to persistent fever and arthritis. She re-presented 2 years later with febrile episodes, rash, arthritis and hepatomegaly and was treated for sJIA flare.

**Patient 6** is a 5-year old male who presented with 6 days of fever and 5/5 KD features. Echocardiography showed no abnormalities. He was treated with IVIG once and high dose ASA, but remained febrile. He continued to have high fevers and on day 15 of his illness he developed desquamation of the skin of his fingers. Subsequently he developed arthritis and despite indomethacin, he continued to have intermittent fevers, rash and arthritis and was diagnosed with sJIA. He required anti-TNF therapy due to ongoing systemic symptoms and continued to have destructive chronic arthritis into adult life.

**Patient 7** is a 5-year-old male who presented with 5 days of fever and 5/5 classic features of KD. Echocardiography showed no abnormalities. He developed arthritis of the hip during this hospital stay. Fevers and arthritis were unresponsive to IVIG (twice) and high dose ASA, requiring high dose IVMP to become afebrile. He continued to have intermittent fever, rash and arthritis. After 3 months he was diagnosed with sJIA and the symptoms ceased with indomethacin. Currently, he is in remission and off medication 11 years post-diagnosis.

**Patient 8** is a 7-year-old male who presented with 5 days of fever and 4/5 principal features of KD. He was treated with IVIG twice and received high dose ASA. Echocardiogram revealed pericardial effusion and CA dilatation which resolved by the 6 weeks. He continued to have recurrent spiking fever, rash and serositis leading to the suspicion of sJIA. Inflammatory arthritis was only observed 4 months later when he fulfilled diagnostic criteria for sJIA. At the last visit, 6 years post-diagnosis, he was in clinical remission and off medication.

## DISCUSSION

Both KD and sJIA have their own distinct set of criteria but share similar clinical manifestations at initial presentation. In our KD

cohort, 0.5% (8/1765) of children had a co-diagnosis of sJIA; whereas KD preceded sJIA in 7% (8/112) of our sJIA cohort. This is higher than what was reported in 2015 where there as a 0.2% incidence of KD/sJIA among 6,745 KD patients based on patient health information database (3). Patients who fulfilled both KD and sJIA criteria had higher leukocyte count, lower albumin levels, and more refractory to standard KD therapy, suggesting a more severe disease at baseline. Conjunctivitis was a less common presenting symptom in KD/sJIA which is similarly observed in other studies (3).

Fever is a major criterion for both disease entities. In KD fever is self-limiting and, if untreated, lasts for 11 days on average but may continue for several weeks while fever in sJIA can have chronic reoccurrence (4). All our KD/sJIA patients experienced prolonged fever occurring more than 6 months after sJIA diagnosis (**Table 2**) but not all exhibiting quotidian fever. This is consistent with reports in sJIA that the classic quotidian pattern occurs in <40% of the patients during initial presentation, followed by 27% with intermittent fevers, 15% with bi-daily fevers, and 5% with persistent fevers (4). Rash and lymphadenopathy are also important criteria for both, but with rash being present in all and lymphadenopathy in half of our KD/sJIA patients, none of these criteria are specific for either KD or sJIA. The clinical resemblance is further highlighted by the presence of arthritis, part of the sJIA criteria, in 7.5% of KD patients (5). KD-associated arthritis, however, occurs in the acute phase of the disease that is characteristically self-limiting or resolves within 2 weeks after IVIG treatment. Although the presumptive diagnosis of sJIA was made before the criterion of 6 weeks of arthritis was met, all KD/sJIA patients subsequently met this criterion during follow-up. The most common joint involved was the knee, followed by the ankle and wrist with equal frequency. KD and sJIA share many clinical inflammatory features that appear to be self-limiting in KD and more prolonged in sJIA.

In addition to arthritis, serositis - particularly pericarditis with associated pericardial effusion - can be a common clinical finding in both. Pericardial effusion had been reported in 5–25% of KD patients (6–8). Echocardiography revealed a higher frequency of pericardial effusion in our KD/sJIA patients (63%), three of whom was detected at the time of KD diagnosis and two shortly thereafter. Pericarditis is seen in 10% of sJIA patients (4). It rapidly responds to anti-inflammatory therapy and does not typically recur with good disease control. Similarly, CA lesions in our KD/sJIA patients resolved within 6 weeks and did not recur. Of the eight KD/sJIA patients, two (25%) developed small coronary artery aneurysm and five (63%) had coronary artery dilatation, all of them in children with incomplete KD. The frequency of CA dilatation was 29–42% (9, 10) in other sJIA series, and it was also noted in several case reports in children from 18 months to 6 years old of varying ethnicities (11–14). It is difficult to estimate the true incidence of CA aneurysm in sJIA because of either unreported or varying z-score cut-off values. Although CA dilatation is a key feature of KD, it may not be as unique to this condition as previously thought. Aside from the coronary artery findings, pericardial and myocardial inflammation are well-known entities that are seen in patients with KD and sJIA. These facts may suggest common mechanism that predisposes to cardiovascular morbidity in inflammatory rheumatic disease.

MAS is a serious complication of childhood rheumatic diseases. It is present in 7–22% of sJIA patients (15, 16) and less commonly seen in KD at approximately 1–2% during initial disease presentation (17–19). In our KD/sJIA patients, 13% (1/8) developed MAS shortly after KD diagnosis. Although we have a small number of KD/sJIA patients, which makes it challenging to interpret, other case series have reported similar increase in this unique patient population by as much as 30% (20). This is important since cardiovascular morbidity is reportedly high when KD is complicated by MAS; the incidence of coronary artery abnormalities in particular is 33–100% (17, 21).

The striking association of sJIA and KD with MAS further points to a potentially common immunobiology. All three entities are syndrome complexes with massive immune activation, similar pro-inflammatory cytokine signatures and clinical response to inhibition of common biologic agents (22–26). Interleukin (IL)-1 beta (β) induces fever, is released from keratinocytes in response to stress or injury (27), and is a key mediator of synovial inflammation. It has been shown that serum from sJIA patients have excessive IL-1β production upon stimulation and triggers IL-1β release peripheral blood mononuclear cells of healthy controls (28). Data from genetic and animal studies in KD implicates a pathogenic role of IL-1β in disease development, response to IVIg and cardiac outcomes (29–31). NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome transcript levels, protein and cellular response are upregulated in KD compared to healthy controls (30). Although the role of IL-1β in MAS is not entirely clear, IL-1β blockade has been effective in the treatment of MAS (15). The hallmark of MAS pathogenesis is the over-production of pro-inflammatory cytokines (IL-1β) by tissue macrophages, which acts through an autocrine mechanism leading to a vicious cycle of further IL-1β production and exaggerated hyperinflammation. It is worth mentioning that tumor necrosis factor alpha (TNFα) inhibitor has been used as second line agent in KD (32, 33). It shortens the duration of the fever and lowers systemic inflammation markers, but its cardio protective effects in patients with refractory KD has not yet been proven. Similarly, treatment with TNFα inhibitors showed transient but un-sustained improvement in sJIA arthritis, its role in controlling fever and other systemic signs has been unsuccessful (34, 35). These observations are important in both diseases and suggests areas for further investigation into underlying pathobiology.

The newly emerged disease entity during the current severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) pandemic called Pediatric Inflammatory Multisystem Syndrome (PIMS) or Multisystem Inflammatory Syndrome in children (MIS-c) presents with similar clinical features and cardiac complications as that of classic KD (36–38). The cytokine storm with marked elevation in serum inflammatory markers resembling sJIA. Patients are managed with IVIg and corticosteroids, and anti-cytokine therapy with anakinra has been used effectively in severe and refractory cases. All these suggests that PIMS/MIS-c is likely part of this IL-1β disease spectrum as well.

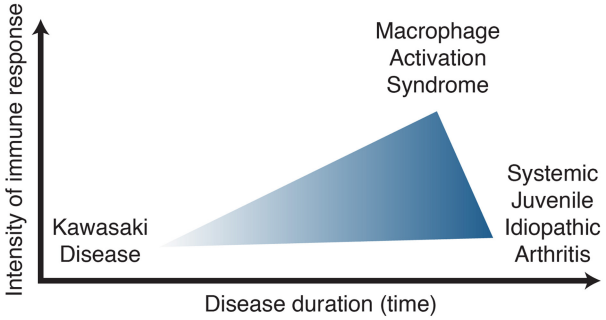


**FIGURE 2 |** Kawasaki disease, systemic juvenile idiopathic arthritis, macrophage activation syndrome spectrum of disease.

Limitation of our work is its retrospective nature and historical nature of the patient cohort. However, we present clinical information and disease outcomes collected in a large number of KD patients over a span of 20 years. It also allowed us to gather follow-up information in KD/sJIA group, with one patient followed-up to more than 10 years. We recognize that the practice patterns in KD and sJIA management have considerably evolved in the last decade with more aggressive use of biologic drugs like IL-1β and TNFα inhibitors, but the ability to make these observations were facilitated by lack of routine anti-cytokine blockage, supporting the observations and conclusions from this paper.

In summary, patients who meet KD criteria should be managed appropriately as such. Prolonged, recurrent fever and rash despite KD treatment should raise suspicion for other systemic inflammatory disorder such as sJIA, especially if arthritis is present. CA dilatation can occur in both disease entities and cannot be used as a sole clinical evidence to differentiate KD and sJIA. Lessons learned from one disease may lead to opportunities for improvement in management. For example, IL-1β blockade in sJIA and MAS may benefit a subset of KD patients with persistent elevation in IL-1β despite IVIg therapy. Alternatively, coronary arteries should be assessed in sJIA patients, and if present, may have implications for therapy, learning from that in KD-associated cardiac disease. Anti-IL-1β treatment may be an effective and more targeted therapy to prevent coronary artery damage in KD, however, more clinical studies are required to know whether it benefits outweigh the risk if used early in those with refractory KD or those with MAS features. Clinical similarities and clues regarding the IL-1β pathway in human and mouse studies suggest common immune biology underlying KD, sJIA and MAS. Thus, we hypothesize that KD, sJIA and MAS are part of the same disease spectrum with the distinguishing features being the intensity and duration of the immune response (**Figure 2**). Further understanding of the immune biology underlying these syndrome complexes will provide better tools to define, manage and improve outcomes of affected children.

## DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Hospital for Sick Children Research Ethics Board. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

EG participated in and reviewed data collection, revised and critically reviewed the manuscript, and approved the final manuscript as submitted. MvV coordinated and supervised data collection, drafted the initial manuscript, and approved the final manuscript as submitted. CM carried out the initial analyses, reviewed the manuscript, and approved the final manuscript as submitted. RS and BM supervised data collection, critically reviewed the report and approved the final manuscript as submitted. RY conceptualized the study, supervised data collection, critically reviewed and revised the manuscript and approved the final manuscript as submitted. All authors contributed to the article and approved the submitted version.

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# MicroRNA-223 Regulates the Development of Cardiovascular Lesions in LCWE-Induced Murine Kawasaki Disease Vasculitis by Repressing the NLRP3 Inflammasome

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Kawasaki disease (KD), an acute febrile childhood illness and systemic vasculitis of unknown etiology, is the leading cause of acquired heart disease among children. Experimental data from murine models of KD vasculitis and transcriptomics data generated from whole blood of KD patients indicate the involvement of the NLRP3 inflammasome and interleukin-1 (IL-1) signaling in KD pathogenesis. MicroRNA-223 (miR-223) is a negative regulator of NLRP3 activity and IL-1β production, and its expression has been reported to be upregulated during acute human KD; however, the specific role of miR-223 during KD vasculitis remains unknown. Here, using the *Lactobacillus casei* cell wall extract (LCWE) murine model of KD vasculitis, we demonstrate increased miR-223 expression in LCWE-induced cardiovascular lesions. Compared with control WT mice, LCWE-injected miR-223-deficient mice (*miR223*$^{-/y}$) developed more severe coronary arteritis and aortitis, as well as more pronounced abdominal aorta aneurysms and dilations. The enhanced cardiovascular lesions and KD vasculitis observed in LCWE-injected *miR223*$^{-/y}$ mice correlated with increased NLRP3 inflammasome activity and elevated IL-1β production, indicating that miR-223 limits cardiovascular lesion development by downmodulating NLRP3 inflammasome activity. Collectively, our data reveal a previously unappreciated role of miR-223 in regulating innate immune responses and in limiting KD vasculitis and its cardiovascular lesions by constraining the NLRP3 inflammasome and the IL-1β pathway. These data also suggest that miR-223 expression may be used as a marker for KD vasculitis pathogenesis and provide a novel therapeutic target.

**Keywords: miR-223, IL-1 beta, Kawasaki disease, vasculitis, NLRP3, LCWE**

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## INTRODUCTION

Kawasaki disease (KD) was first reported by Tomisaku Kawasaki in Japan, in 1967. KD is a systemic vasculitis and myocarditis of unknown etiology and is the leading cause of acquired heart disease in children in developed countries (1). KD has been reported worldwide; however, its incidence is 10 to 30 times higher among children living in Asian countries or from Asian ancestry compared to those in Europe or in the United States (1–3). Untreated KD can lead to the development of coronary artery aneurysms (CAA) in up to 30% of patients (4). KD treatment consists of a single dose of IVIG (2 g/kg) and aspirin, which reduces the risk of CAA development to ≈4% (1). However, up to 15–20% of IVIG-treated KD patients do not respond to the treatment; these patients develop persistent fevers within 36 to 72 h after the end of IVIG infusion and are at increased risk for developing CAA (5–7). Several alternative treatments such as IL-1 receptor antagonist (anakinra) (8–10), anti-TNF-α antibody (11), corticosteroids (12, 13), and calcineurin inhibitors like cyclosporine (14) have been successfully used to treat IVIG-resistant patients (15).

The NLRP3 inflammasome and IL-1β production have a crucial role in KD pathogenesis. Peripheral blood mononuclear cells (PBMCs) isolated from KD patients spontaneously secrete IL-1β (16), and serum levels of IL-1β are higher during acute KD and decrease markedly during the convalescent phase (17, 18). The expression of IL-1-related genes is also upregulated in PBMCs isolated from KD patients during the acute phase of illness (19, 20), and increased transcript abundance of several genes from the IL-1 pathway is associated with IVIG-resistance (21). Genome-wide association studies led to the identification of single-nucleotide polymorphisms (SNPs) associated with increased susceptibility to KD linked to NLRP3 inflammasome activation and IL-1β production (18, 22, 23). Studies in experimental murine models of KD vasculitis have confirmed those observations and further demonstrated the deleterious role of NLRP3 overactivation and enhanced IL-1β production during KD. Blocking the IL-1 pathway genetically using either *Il1β*$^{-/-}$, *Il1r1*$^{-/-}$, or *Nlrp3*$^{-/-}$ mice, or treating WT mice with either IL-1α- or IL-1β-neutralizing antibodies or IL-1 receptor antagonist, anakinra, significantly reduces vasculitis in the *Lactobacillus casei* cell wall extract (LCWE)-induced KD model (24–26). Similar results were reported with the Candida albicans water-soluble fraction (CAWS) murine model of KD vasculitis (27, 28).

MicroRNAs (miRNAs) are small (20 to 23 nucleotides), endogenous, non-coding RNA molecules that are responsible for various cellular and metabolic pathways, including cell proliferation, differentiation, and death (29). In addition, miRNAs are also involved in the regulation of inflammatory responses and the maintenance of immune homeostasis (30, 31). Indeed, miRNAs control the expression of targeted proteins by either inhibiting mRNA translation or decreasing the levels of their corresponding mRNA (32, 33). Among immune regulatory miRNAs, miR-223, initially identified as specific to the hematopoietic lineage (34), inhibits NLRP3 inflammasome activation in acute lung injury (35) and regulates intestinal inflammation (36). In human KD studies, miR-223 expression is upregulated in KD patients' plasma (37) and whole blood (38), as well as in the coronary artery tissue from autopsy samples collected from children with KD (39).

Although local and systemic miR-223 expression is upregulated during human KD, whether miR-223 prevents or promotes the development of KD coronary arteritis still remains unknown. The objective of this study was to characterize the contribution of miR-223 to the development of LCWE-induced cardiovascular lesions using the well-accepted LCWE-induced murine model of KD vasculitis. We demonstrate that miR-223 expression is upregulated in inflamed abdominal aortic aneurysms and dilatations of LCWE-injected mice. Mice genetically deficient in miR-223 exhibit markedly exacerbated heart vessel inflammation and abdominal aorta aneurysm development, as well as increased levels of circulating IL-1β. Overall, our results support the concept that upregulation of miR-223 in inflamed tissues is beneficial and acts as a feedback mechanism to control the pathological and deleterious overactivation of the NLRP3 inflammasome and subsequent IL-1β production.

## MATERIALS AND METHODS

### Mice

Wild-type (WT) C57BL/6 and *miR223*$^{-/y}$ (B6.Cg-*Ptprc*$^{a}$ *Mir223* $^{tm1Fcam}$*/J*) mice were purchased from Jackson Laboratories. For this study, we only used male animals, as LCWE injection induces more severe and more consistent coronary vasculitis lesions and abdominal aorta aneurysms in male than female mice (25, 40). All animals were housed under specific pathogen-free conditions at the animal center of Cedars-Sinai Medical Center. Experiments were conducted under approved Institutional Animal Care and Use Committee protocols.

### Preparation of LCWE

LCWE was prepared as previously described (24). Briefly, *Lactobacillus casei* (ATCC 11578) was grown in Lactobacillus de Man, Rogosa, and Sharpe broth (EMD Millipore, MA, USA) for 48 h, harvested, and washed with PBS. The harvested bacteria were disrupted by an overnight treatment with two packed volumes of 4% SDS/PBS. Cell wall fragments were washed with PBS, and SDS-treated cell wall fragments were sonicated for 2 h with a 3/4-inch horn and a garnet tip at maximum power. During sonication, the cell wall fragments were kept in a dry ice/ethanol bath. After sonication, cell wall fragments were spun for 20 min at 12,000 rpm and 4°C. The supernatant was centrifuged for 1 h at 38,000 rpm and 4°C, and the pellet was discarded. The total rhamnose content of the cell wall extract was determined by a colorimetric phenol-sulfuric assay as described previously (41).

### LCWE-Induced KD Mouse Model

Five-week old male mice were injected intraperitoneally with 500 μg of LCWE (total rhamnose amount as determined above) or PBS. Two weeks later, mice were euthanized and hearts removed and embedded in optimal cutting temperature compound (OCT) for histological examination. For abdominal aorta, diameters were measured at five different parts (below the left renal artery)

and maximal abdominal diameters were calculated. In some experiments, aortas were kept in RNA later for RNA extraction. Serial sections (7 μm) of heart tissues were H&E stained and used for pathological examination. Only sections that showed the second coronary artery branch separating from the aorta were analyzed. Histopathological examination and heart vessel inflammation score (coronary arteritis, aortic root vasculitis, and myocarditis) were performed by a pathologist (M.C.F.) blinded to the genotypes or experimental groups, as described previously (24). All images were acquired either with a Biorevo BZ-9000 or BZ-X710 (Keyence) and were further analyzed with ImageJ software.

## RNA Isolation and Quantitative Real-Time PCR

Freshly dissected aortas were stored in RNA later (Qiagen) before RNA extraction. RNA extraction was performed using the miRNEasy micro kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qPCR) for miR-223 was performed using the Power SYBR Green RNA-to-Ct 1 step kit according to the manufacturer's instructions (Thermo Fisher Scientific) with the following primer sequences: 5'-TGTCAGTTTGTCAAATACCCCA-3' and 5'-GCGAGCACAGAATTAATACGAC-3', as previously published (42).

## ELISA

IL-1β in serum samples was measured using the U-PLEX Mouse IL-1β Assay (Meso Scale Diagnostics) per the manufacturer's instructions. The samples were read and analyzed by MSD QuickPlex SQ120 instrumentation and Workbench 4.0 Software (Meso Scale Diagnostics).

## Statistical Analysis

Results are reported as mean ± SEM. All data were analyzed using GraphPad Prism software. Statistical significance was evaluated by Student's *t* test (two-tailed) to compare unpaired samples between experimental groups. In experiments where data was not normally distributed, the Mann–Whitney test was performed. A probability value of $<0.05$ was considered statistically significant.

# RESULTS

## miR-223 Expression Is Increased During LCWE-Induced KD Vasculitis

As compared with PBS-injected WT control mice, LCWE injection resulted in the development of heart inflammation manifested by aortitis and coronary artery aneurysms (**Figures 1A,B**). LCWE-injected mice also exhibited prevalent abdominal aorta aneurysms and dilatations (**Figures 1C,D**). Elevated levels of miR-223 have been reported in the serum of acute KD patients (43), and miR-223 expression is increased in coronary artery aneurysms of KD children. To assess miR-223 expression in the mouse model, we isolated RNA from the abdominal aortas of control mice and LCWE-injected KD mice and measured miR-223 expression by quantitative PCR. Compared with PBS-injected control mice, miR-223 expression levels were significantly higher in abdominal aorta aneurysms of LCWE-injected mice (**Figure 1E**).

## Exacerbated LCWE-Induced Cardiovascular Lesions in miR-223$^{-/y}$ Mice

To determine the effect of miR-223 on the development of cardiovascular inflammation, we injected WT or *miR223*$^{-/y}$ mice with either PBS or LCWE and assessed the severity of LCWE-induced KD vasculitis 2 weeks later. Compared with WT mice, LCWE-injected miR-223-deficient mice showed significantly more severe heart vessel inflammation and coronary arteritis (**Figures 2A,B**). Similarly, deletion of miR-223 also resulted in enhanced development of abdominal aorta aneurysms (**Figure 3A**) and greater aortic dilations and maximal abdominal aorta diameter (**Figure 3B**). miR-223-deficient mice injected with PBS did not have any cardiovascular lesions and were similar to the WT mice injected with PBS (data not shown). Overall, our results strongly indicate that miR-223 is required to control the severity and development of LCWE-induced cardiovascular lesions, and miR-223 deletion results in worsened heart and abdominal aorta inflammation.

## miR-223 Regulates LCWE-Induced Vasculitis Induction by Regulating IL-1 Production

LCWE-induced KD vasculitis is NLRP3 dependent, and it has been previously shown that miR-223 regulates NLRP3 activation and IL-1β production (36). To further determine if miR-233 dampens LCWE-induced KD vasculitis in WT mice by decreasing NLRP3 inflammasome activation and subsequent IL-1β production, we next quantified the circulating levels of IL-1β in LCWE-injected WT and *mir-223*$^{-/y}$ mice 1 week after LCWE injection. In agreement with our observation of heightened heart vessel inflammation and increased severity of abdominal aorta aneurysms in LCWE-injected *miR223*$^{-/y}$ mice (**Figures 2**, **3**), circulating IL-1β was markedly higher in the absence of miR223 (**Figure 4**). Overall, our results indicate that miR-223 does not completely prevent the development of LCWE-induced cardiovascular lesions; however, by restraining NLRP3 activation and its subsequent IL-1β production, miR-223 appears to curtail vascular tissue inflammation.

# DISCUSSION

MicroRNAs (miRNAs) are critical regulators of a host of cellular processes, including inflammation (30, 31). These endogenous, non-coding, single-stranded RNAs of 20–23 nucleotides exert regulatory functions through complementary base pairing to the 3′ untranslated regions (UTRs) of protein-coding mRNAs. Many immune processes are regulated by miRNA-mediated RNA interference, but how miRNA circuits orchestrate aberrant cardiovascular inflammation during KD vasculitis is poorly defined. The role of miRNAs in regulating innate immune responses has primarily been investigated for TLR signaling (44). Indeed, several miRNAs were identified as induced following

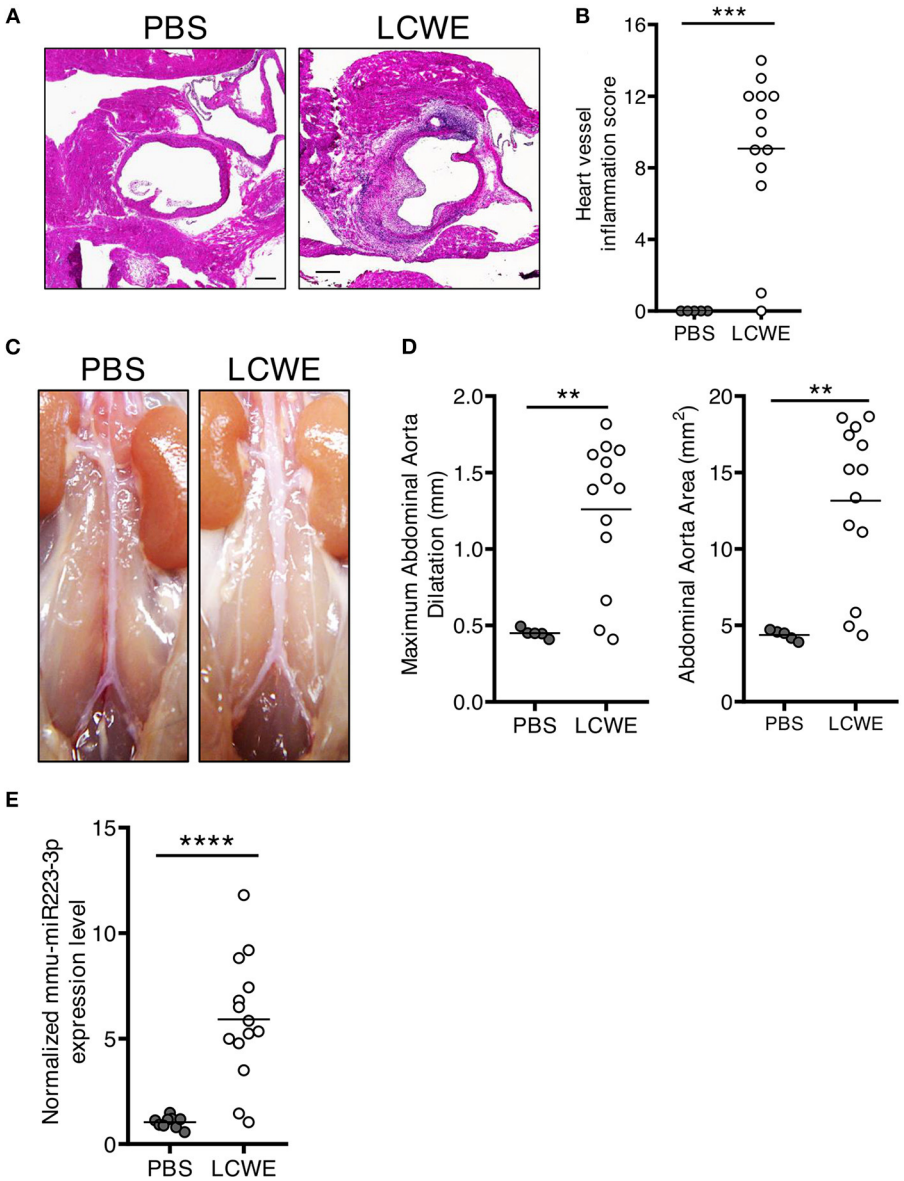


**FIGURE 1 |** Increased miR-223 expression during LCWE-induced KD vasculitis. Five-week-old mice were injected i.p. with either PBS or LCWE, and 2 weeks later vasculitis severity was assessed. **(A)** H&E staining of heart tissue sections from PBS and LCWE-injected WT mice. **(B)** Heart vessel inflammation score of PBS and LCWE-injected WT mice. **(C)** Pictures of the abdominal aortas of PBS-injected and LCWE-injected mice. **(D)** Maximal abdominal aorta diameter and abdominal aorta area of PBS and LCWE-injected mice 2 weeks post-LCWE injection. **(E)** miR-223 mRNA quantification in the abdominal aorta of PBS and LCWE-injected mice. $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$ by unpaired *t*-test.

TLR activation, targeting mRNAs encoding components of the TLR-signaling pathway itself. These regulatory systems likely evolved to allow a strong initial immune response that must be gradually dampened down after the secondary induction of the regulatory miRNAs. miRNA-223 is a critical regulator of NLRP3 inflammasome activity (42). miR223 suppresses NLRP3 expression through a conserved binding site within the 3′ untranslated region of NLRP3, resulting in reduced NLRP3 inflammasome activity (42). Thus, miR-223 functions as an additional layer of control beyond the tight transcriptional control of NLRP3 message itself. miR-223 also plays a key role in the regulation of granulocyte differentiation, among other functions (34).

Changes in the expression of multiple miRNAs are reported during KD; however, whether these differentially expressed miRNAs have a beneficial or deleterious role on KD pathogenesis remains unknown. Shimizu et al. reported that six microRNAs (miRs-143,−199b-5p,−618,−223,−145, and−145) were significantly elevated in whole blood of acute KD patients (38). Elevated miR-145 expression in blood samples from

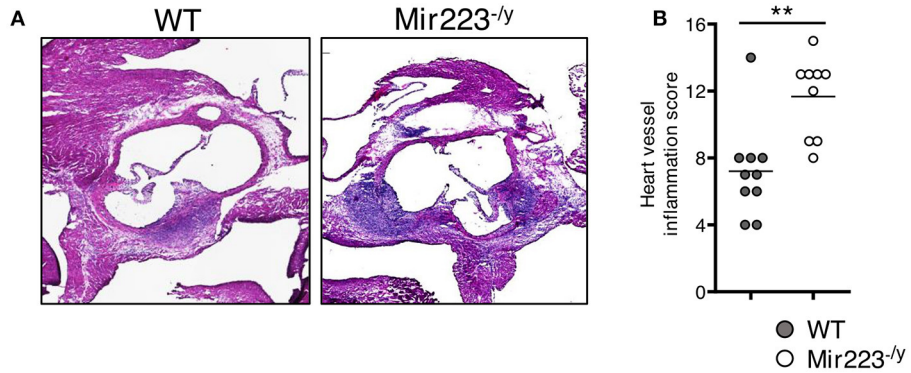


**FIGURE 2 |** Deletion of miR-223 promotes the development of LCWE-induced vasculitis. Five-week-old male WT and *miR-223*$^{-/y}$ mice were i.p. injected with LCWE, and 2 weeks later the severity of vasculitis was assessed. **(A)** H&E heart tissue sections of LCWE-injected WT and *miR-223*$^{-/y}$ mice. **(B)** Heart vessel inflammation score of the mice group **(A)**. $^{**}p < 0.01$ by unpaired *t*-test.

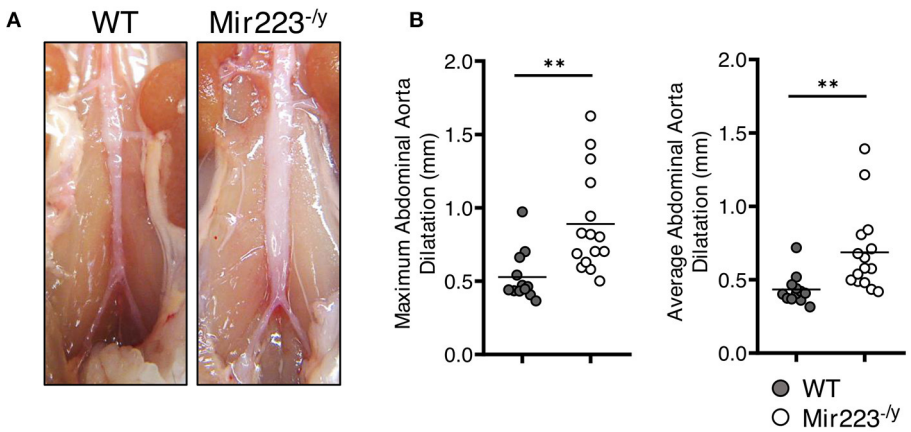


**FIGURE 3 |** Deletion of miR-223 promotes the development of LCWE-induced abdominal aortitis. Five-week-old male WT and *miR-223*$^{-/y}$ mice were i.p. injected with LCWE, and 2 weeks later the severity of vasculitis was assessed. **(A)** Abdominal aorta pictures of LCWE-injected WT and *miR-223*$^{-/y}$ mice. **(B)** Maximal abdominal aorta diameter and average abdominal aorta diameter from WT and *miR-223*$^{-/y}$ mice injected with LCWE. $^{**}p < 0.01$ by unpaired *t*-test.

acute KD patients was further confirmed by qRT-PCR in an independent cohort. miR-145 may play a critical role in the differentiation of neutrophils and vascular smooth muscle cells by modulating TGF-β signaling in the arterial wall (38). Yun et al. reported that the serum levels of miRNA-200c and miRNA-371-5p were elevated during KD and could potentially be used as a KD diagnostic biomarkers (45). Rong et al. suggested that miR-27b could affect endothelial cell proliferation and migration *via* targeting Smad7 and affecting TGF-β pathway. Thus, miR-27b may be a potential biomarker for KD and a therapeutic target for KD treatment (46). In another study, seven miRNAs were significantly upregulated (hsa-let-7b-5p, hsa-miR-223-3p, hsa-miR-4485, hsa-miR-4644, hsa-miR-4800-5p, hsa-miR-6510-5p, and hsa-miR-765) and three were significantly downregulated (hsa-miR-33b-3p, hsa-miR-4443 and hsa-miR-4515) in acute KD compared with the healthy controls by miRNA microarray analysis (37). Of these miRNAs, miR-223 was consistently detected by RT-qPCR (37), and previous studies have reported that miR-223 may regulate inflammation of vascular endothelial cells (47).

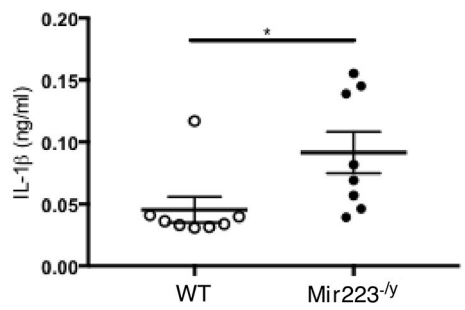


**FIGURE 4 |** miR-223 dampens LCWE-induced vasculitis induction by regulating IL-1β signaling. IL-1β quantification in the serum of WT and *miR-223*$^{-/y}$ at 1 week post-LCWE injection. $^{*}p < 0.05$ by unpaired *t*-test.

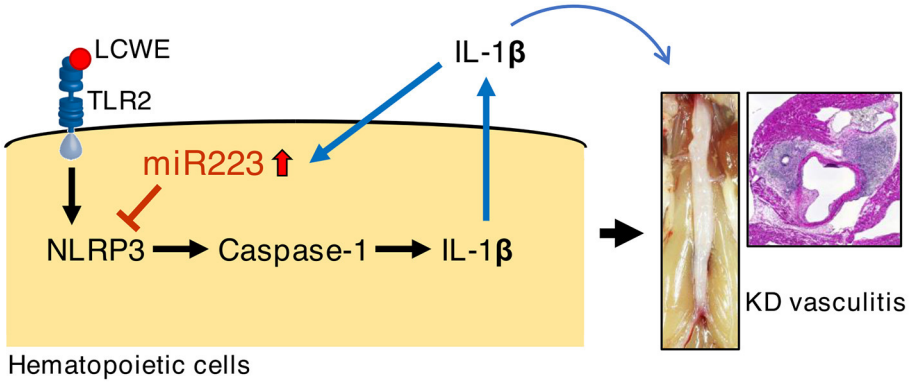


**FIGURE 5 |** Possible mechanism of miR-223 regulation during LCWE-induced vasculitis.

The role of miR-223 in regulating innate immune response and inflammation is increasingly appreciated. Absence of miR-223 exacerbates inflammation in a murine model of colitis characterized by enhanced NLRP3 inflammasome activation and IL-1β production (36). LPS and poly(I:C) activation decrease miR-223 expression in macrophages through TLR4 and TLR3. In turn, downregulation of miR-223 promotes TNF-α, IL-6, and IL-1β production upon LPS stimulation (42, 48). miR-223 has also been shown to disrupt NLRP3 inflammasome activity in a mouse model of hepatitis (49). In acute gout patients, colchicine can upregulate miR-223-3p and downregulate IL-1β in the plasma (50). LPS can reduce miR-223, while promoting the production of IL-1β in human adipose stem cells *via* TLR2 (51). Previously, we reported that macrophages infiltrate the cardiovascular lesions of LCWE-injected mice (25, 26). These infiltrating macrophages show high caspase-1 activity by FLICA staining (26). In this study, we found that miR-223 was highly expressed in the abdominal aorta of LCWE-injected mice. Moreover, *miR-223*$^{-/y}$ mice showed more severe abdominal aorta dilatations and heart vasculitis and higher concentrations of serum IL-1β, which is a key mediator of LCWE-induced KD vasculitis (24, 25). We hypothesize that intense IL-1β production during LCWE-induced KD vasculitis induces miR-223 expression as a negative regulatory feedback loop to suppress IL-1β function and further development of vasculitis (**Figure 5**).

Johnnidis et al. reported that miR-223 negatively regulates granulocyte differentiation and dampens neutrophil activation and effector functions (34). Indeed, *miR-223*-deficient mice spontaneously develop lung pathologies due to increased numbers of granulocyte progenitors and neutrophils, which are more easily activated (34). Additionally, Dorhoi et al. reported that in an experimental murine model of *Mycobacterium tuberculosis* infection, miR-223 deletion resulted in exacerbated neutrophil-driven lung inflammation (52). We have reported that Ly6G$^{+}$ neutrophils infiltrate the abdominal aorta aneurysm area of LCWE-injected WT mice (25), and similar results were also observed with the CAWS murine model of KD vasculitis, where a massive influx of Ly6G$^{+}$ neutrophils is detected in the coronary artery of WT mice 28 days after CAWS injection (53). Since miR-223 regulates neutrophil differentiation and activity, it is possible that hypersensitive and activated neutrophils participate and enhance the severity of cardiovascular lesions observed in LCWE-injected *miR-223*$^{-/y}$ mice; however, future studies will be needed to investigate the role of miR-223 in modulating neutrophil activity in this experimental model. In addition, human platelets also express significant amounts of miR-223, and platelet miR-223 levels are important for their reactivity (54). The lower expression of miR-223 may increase platelet reactivity and the risk of thrombotic disease, such as myocardial infarction (55). In the acute stage of KD, patients show thrombosis and decreased platelet numbers, and then thrombocytosis is consistently found in the 2nd to 3rd week of illness (56). Lack of platelet-derived miR-223 in KD patients may increase the risk of coronary artery pathology (43). Recent studies have also shown that leukocytes and platelet-secreted miR-223 can enter vascular smooth muscle cells, and appear to play important protective roles in their function in experimental models of atherosclerosis (57), in an arterial injury repair model (58), and in a KD mouse model by decreasing VSMC proliferation (59).

Although miR-223 has been reported to negatively control NLRP3 activation (42), it is also possible that miR-223 targets other proteins involved in inflammatory immune responses that may potentially contribute to LCWE-induced KD vasculitis, such as CXCL2, CCL3 (52), STAT3, and IL-6 (60). While we hypothesize that in this model miR-223 affects the NLRP3 and IL-1β pathway, the specific mechanisms of this regulation will need to be further demonstrated in future experiments.

## CONCLUSIONS

Collectively, our studies highlight the miR-223-NLRP3-IL-1β regulatory circuit as an important component of vascular inflammation development in the experimental LCWE-induced murine model of KD vasculitis. Our data reveal a previously unappreciated role of miR-223 in regulating the level of NLRP3 inflammasome activation and shows that miR-223 provides an

early break, limiting IL-1β-mediated vascular inflammation in LCWE-injected mice. NLRP3 is under a tight transcriptional control, and miR-223 has been shown to work as an important rheostat controlling NLRP3 inflammasome activation (42). Our observation that miR-223 deficiency results in a more severe LCWE-induced KD vasculitis is therefore consistent with this previous report and indicates that a similar control of inflammation may indeed occur in the LCWE-induced KD murine model. Hence, miR-223 may be a potential biomarker for early diagnosis of human KD, and as miR-223 can dampen cardiovascular inflammation, pharmacologic stabilization of miR-223 may hold promise as a future novel therapeutic modality for KD.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

All experiments were performed according to Cedars-Sinai Medical Center Institutional Animal Care and Use Committee (IACUC) guidelines.

## AUTHOR CONTRIBUTIONS

MAr conceived and supervised the project. MAr, YL, and MNR wrote the manuscript. DMa, BK, YL, MAb, ML, DMo, SC, MCF, and RAP performed all of the experiments. SC, RAP, MNR, and MAr provided critical editing and content to the manuscript as well as experimental design. All authors read and approved the final manuscript.

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MINI REVIEW
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# Epidemiology of Kawasaki Disease in Europe

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**Aim of the review:** To review major epidemiological aspects of Kawasaki disease (KD) in Europe, describing demographic characteristics, revising its incidence along with time trends and geographic variations, and describing migration studies to provide clues about its etiology.

**Recent findings:** The annual incidence of KD in Europe is about 10–15 per 100,000 children under 5 years old and seems to be relatively stable over time and space. Demographic characteristics are in line with those in other countries of the world, with a higher incidence in children from Asia and possibly North African origin. All studies performed across Europe found a coherent seasonal distribution of KD onset peaking from winter to early spring. This seasonal distribution was consistent over the years and suggests a climate-related environmental trigger. The occurrence of peaks during pandemics, microbiological findings and a possible link with southerly winds support the hypothesis of an airborne infectious agent. Neither other airborne agents such as pollutants or pollens nor urbanization and industrialization seem to have major effect on the etiology.

**Conclusion:** Discrepancies in KD incidence rates across studies were due more to methodological differences, variation in definitions and awareness of the disease than a real increase in incidence. Genetic predisposition is undeniable in KD, but environmental factors seem to play a pivotal role. Several lines of evidence support a non-exclusive airborne infectious agent with a protective immune response by the host as a key factor in inducing the inflammatory cascade responsible for symptoms and complications.

**Keywords: Kawasaki disease, vasculitis, epidemiology, incidence, children, Europe, coronary arterial lesions**

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## INTRODUCTION

Kawasaki disease (KD) is the main systemic vasculitis of children under 5 years old, affecting predominantly medium-size arteries, particularly coronary arteries. This acute disease, described in 1967 by Tomisaku Kawasaki, is the leading cause of acquired heart disease in childhood in developed countries (1). The disease is described in all continents but with variable frequency. The highest annual incidence is in Asian countries, and the latest published rate (per 100,000 children

under 5 years old) seems to progressively decrease from 359 in Japan, 197 in South Korea, and 95 in Shanghai to 75 in Taiwan (2–5). In non-Asian countries, the annual incidence is about 10–20 per 100,000 children under 5 years old, as described in North America, Europe, Chile or Australia (6, 7). In Hawaii, the higher incidence rate of 32 per 100,000 compared to the rest of the United States is due to the large number of children with an Asian origin (8). Little is known about the impact of KD in Africa, South America, and the Near and Middle East.

Despite extensive research, the exact cause of KD remains unknown. Important clues can be obtained by epidemiological investigations searching for etiologic factors. Disparities in incidence between countries coupled with migration studies showing a higher incidence for children with an Asian origin than Caucasian children support a genetic predisposition to the disease (8, 9). Moreover, genome-wide association studies have described polymorphisms associated with the occurrence of KD or complications (10, 11). However, genetics does not explain all of the epidemiological characteristics of the disease. Family forms are rare (2% in siblings, 1% in parents) (2). Many countries have reported an increased incidence of KD over the last decades (2–5, 12). Whether this increase is real or due to increased physician awareness is unclear. In South Korea, Shanghai and Taiwan, incidence rates seem to have stabilized in the last years (3–5). In Japan, where there is a long-standing widespread physician awareness of the disease, the increased incidence might be due to a true increase in case numbers. However, nationwide KD surveys to monitor KD every 2 years since 1970 throughout Japan showed an increased number of patients with incomplete KD without coronary artery abnormalities in the last 4 decades (2). Therefore, the increased number of patients with incomplete KD without such abnormalities could be due to better recognition of the incomplete form, a real increase of incidence or even an over-diagnosis.

Three epidemic peaks in Japan and the seasonal variation in KD support the hypothesis of an environmental factor as a trigger for KD. However, seasonal variation differs among countries. Some countries reported peaks of incidence in winter (Japan, Canada) or in spring (Taiwan) and others in summer (Korea) or autumn (India, Costa Rica) or no seasonality (Hawaii) (8, 13). Results were in agreement for seasonality in the northern hemisphere, with highest number of cases in January through March, which suggests an infectious agent operating during winter months (14). Results for a link with rainfall were discordant (13). Analyses have correlated the incidence of KD cases in Japan, Hawaii, and San Diego with tropospheric wind patterns originating from northeastern China, which suggests that a wind-borne agent, possibly a fungal toxin from agricultural sources, could trigger the illness (15). In addition, intracytoplasmic inclusion bodies found in ciliated bronchial epithelium in three dead patients suggested a viral air-borne agent (16). However, the role of a defective intestinal barrier function in the development of KD is under investigation. Increased intestinal permeability with elevated serum immunoglobulin A (IgA) level and changes in intestinal microbiota composition have been described in patients with KD (17). Age of disease onset, self-limited course of this acute disease, and low rate of recurrence suggest an environmental factor, with the host inducing a protective immune response. Bacterial, fungal and viral agents have been incriminated, but none is constantly present or universal, which suggests that non-specific agents trigger the inflammatory cascade, as observed in auto-inflammatory diseases. Other environmental agents such as toxins, food, and living standards may have modulatory effects (11).

This review aims to describe the major epidemiological aspects of KD in Europe, describing demographic characteristics, revising its incidence along with time trends and geographic variations, and describing migration studies in comparison with other geographic areas to provide clues about its etiology.

## INCIDENCE RATES

**Figure 1** illustrates the incidence rates of KD in European countries. Most of the studies were performed in northern and western European countries, and little is known about the epidemiology of KD in eastern European countries. The range of incidence rate is broad, with a minimum of 1.6 per 100,000 children under 5 years old in the Czech Republic and a maximum of 17.6 in Italy (18, 19). However, the period of the studies and methodological differences could explain these differences. An increased awareness of the disease in the last 20 years combined with a better definition of cases after publication in 2004 of The American Heart Association (AHA) classification criteria for KD have progressively improved the diagnosis of KD (20).

The incidence of 1.6 per 100,000 children under 5 years old in the Czech Republic comes from a study performed between 1997 and 1999, when many physicians were not aware of KD (18). Moreover, the study was prospective, with a monthly mailed questionnaire sent to heads of pediatric departments. Such voluntary reporting by busy physicians can under-record cases. The United Kingdom has the largest number of studies on the incidence of KD: annual estimates ranged from 3.4 to 15.2 per 100,000 children under 5 years old. In 1990, the incidence in the United Kingdom and Ireland was 3.4 per 100,000 children, but the period and study design were similar to the study performed in the Czech Republic and probably underestimated the true incidence (21). In 1996, using a more accurate methodology with multiple sources of case identification, Gardner-Medwin et al. found an annual incidence of 5.5 per 100,000 children in the West Midlands (England) (22). A capture–recapture analysis estimated that case finding might have missed 40% of cases. The same year in Ireland, Lynch et al. described an average annual KD hospitalization rate of 15.2 per 100,000 children (23). Because a child could be hospitalized in several hospitals or several times during the same episode, this rate was an overestimation of the true incidence. In 1998, before publication of the AHA criteria and increased awareness of KD by physicians, Harnden et al. found an incidence of 8.4 per 100,000 children by using administrative data (24). Recently, Hall et al. found an incidence of 9.1 per 100,000 children by using The Health Improvement Network database of electronic primary healthcare records from practices throughout the United Kingdom. This database has

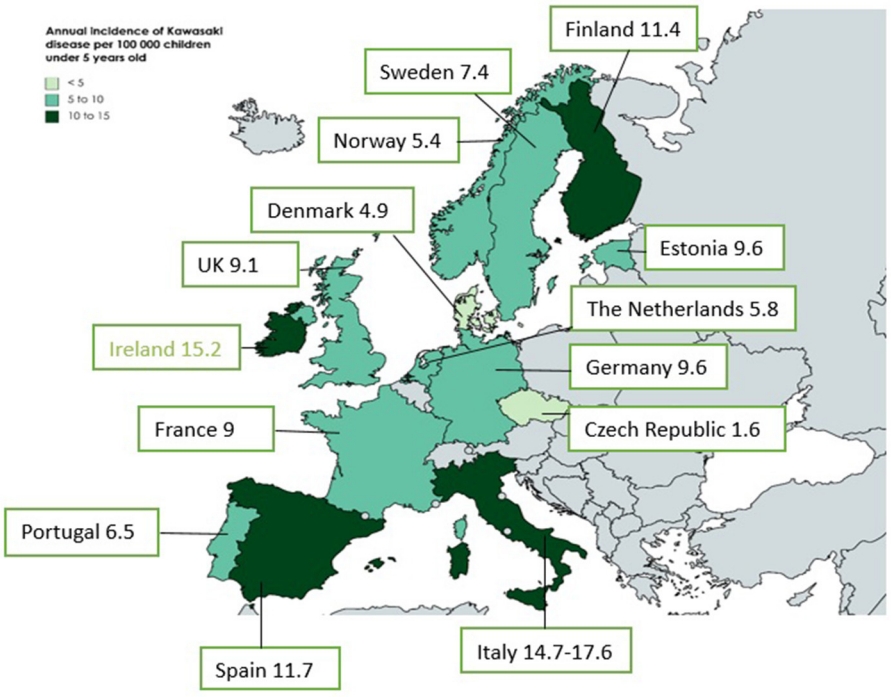


**FIGURE 1 |** Annual incidence rates of Kawasaki disease in European countries. The number in green represents the hospitalization rate in Ireland.

been shown to be generalizable to the UK population although with slightly fewer people aged <20 years as compared with the general UK population (25). Finally, from 2013 to 2015, Tulloh et al. found an annual incidence of 4.6 per 100,000 children with the same methodology used in 1990 and obtained a probable underestimation of the true incidence (26). The slight increase in incidence as compared with 1990 is probably due to increased awareness in the last years and because the authors contacted pediatric cardiologists in addition to pediatricians. These observations underline the importance of the methodology in interpreting results. European studies based on hospital discharge data published in the last 10 years showed annual incidence rates of 9.1–14.7 per 100 000 children under 5 years old (19, 25, 27, 28), whereas those based on prospective surveillance found lower rates (26, 29) (**Table 1**). A prospective surveillance design in Germany with cross validation with hospital records from two states found that the hospital-based German Pediatric Surveillance Unit (ESPED) missed 37–44% of cases. For all these reasons, the incidence of KD in Europe is probably more 10–15 per 100,000 children under 5 years old.

## ETHNICITY AND MIGRATION STUDIES

Similar to US studies, migration studies performed in the United Kingdom and Spain showed a higher incidence for children with an Asian origin than white peers (22, 24, 31). In England, a significant association was found between Chinese ethnicity and incidence of KD, peaking at 13.6 instead of 6.6 in areas with more than 1% of the population of Chinese ethnicity as compared with areas with low (<0.18) population of Chinese ethnicity (24). A weaker association was found between KD incidence in areas of high proportion of black ethnicity (24). In West Midlands, the incidence was 3-fold higher for Asian than white children. However, no children were Chinese, Japanese, or from other countries in the east of the Indian subcontinent but rather were of Indian, Pakistani, or Bangladeshi origin. In Spain, KD was more common in Asian and North African children (31).

## TIME TRENDS

All studies performed across Europe found a coherent seasonal distribution of KD onset peaking from winter to early spring (24, 26, 29, 30, 32–34). This seasonal distribution was consistent over the years (24, 26, 32) and suggests a climate-related environmental trigger.

The incidence of KD seemed to remain relatively stable over time in Europe. In Denmark, the incidence progressively increased from 1980 to 1999 owing to increased awareness by physicians, with better diagnosis and an increased tendency for hospitalization, but the incidence has stabilized since 1999 (32). From 2000 to 2011, KD cases ranged from 30 to 50 per year in Portugal, with a mean of 39 cases per year (35). The

**TABLE 1 |** Reported annual incidence rate (per 100,000 children under 5 years old) in the last 10 years in European countries.

| References | Country | Study period | Source of case identification | No. of cases | Annual incidence rate |
|---|---|---|---|---|---|
| Cimaz et al. (19) | Italy | 2008–2013 | National hospital discharge record database | 2,901 | 14.7 |
| Riancho-Zarrabeitia et al. (27) | Spain | 2005–2015 | Hospital morbidity survey of the Spanish National Institute of Statistics (INE) database | 3,737 | 11.7 |
| Piia Jogi et al. (28) | Estonia | 2008–2019 | Hospital discharge records | 85 | 9.6 |
| Hall et al. (25) | United Kingdom | 2008–2012 | The Health Improvement Network (THIN) database | 109 | 9.1 |
| Tulloh et al. (26) | United Kingdom+ Ireland | 2013–2015 | Prospective surveillance | 553 | 4.6 |
| Tacke et al. (29) | The Netherlands | 2008–2012 | Prospective surveillance | 341 | 5.8 |
| Jakob et al. (30) | Germany | 2011-2012 | Prospective national surveillance (ESPED) + cross-validation with hospital record data in 2 federal states | 272 | 7.2–9.6 |

annual incidence was relatively stable from 2008 to 2012 in The Netherlands, from 2008 to 2013 in Italy and from 2005 to 2015 in Spain (19, 27, 29). A study performed from 1981 to 2013 in the University hospital of Lausanne in Switzerland described an increase in number of KD cases since 1981. According to the authors, this increase resulted from better diagnosis of KD due to a better recognition of incomplete forms. Besides the increased awareness of the disease with time, the authors mentioned that the recent increase could be due to an improvement in imaging modalities with better diagnosis of cardiac complications. They also underlined that in more than 50% of patients, z-score calculations changed the degree of coronary artery involvement from the initial echocardiographic report, which had underestimated the coronary artery lesions in many cases (34). Indeed, using absolute dimension of coronary arteries could underestimate prevalence of anomalies as size of coronary arteries vary with age and body surface (36). Z-scores normalize coronary artery luminal dimension for body surface area and allow comparison across time and populations (1). However, several formulas for z-score calculation have been described yielding discrepant z-scores, which might affect diagnosis and clinical decisions (1, 36). Therefore, changes in incidence in Europe seem to be related more to increased awareness by physicians, improved imaging modalities, and modification in classification criteria (AHA and z-score calculation) than to a real increase in incidence.

## DEMOGRAPHIC CHARACTERISTICS

Although KD can occur in young adults and children of all ages, the disease has a pronounced predilection for children 1–5 years old (37–39). In Italy, the annual incidence in children under 15 years old was 5.7 per 100,000 but peaked at 14.7 in children under 5 years old (19). In a Spanish series of 625 children under 16 years old with a diagnosis of KD, 79% of cases were younger than 5, 16.5% younger than 1 and 6.7% younger than 6 months (40). The reported mean age of onset varies from 1.9 to 2.8 years, but the youngest patients could be as young as 1 month old (21, 26, 29, 30, 32–35, 40–43). Most studies, except one with very few patients in Austria, found a male predominance, with male-to-female ratios of 1.3–1.8 (19, 21, 24, 26, 29, 30, 33–35, 40–42, 44). In recent years, 58–80% of KD cases were complete as compared with 80–87% in the 1990's (21, 30, 42, 43, 45). The mean delay to treatment with intravenous Ig was 5.3–10 days (26, 29, 34, 40, 42), mostly about 7 days (21, 46, 47). Overall, 11% to 23% of children showed resistance to the first intravenous Ig infusion (29, 34, 40, 42, 46, 47). Frequencies of coronary artery lesions ranged from 2 to 65% (19, 21, 26, 34, 35, 40, 42, 46–48). This discrepancy is due to variations in definition and assessment of coronary artery involvement, time of evaluation and study design. The disease was recurrent in < 2.5% of cases (34, 35, 40, 43) and mortality <0.5% in recent series (19, 26, 29, 34, 35, 40, 42, 44, 47). Familial cases are rare, reported in <1% of cases in first- and second-degree relatives (40). **Appendix 1** reports the main clinical characteristics of children with KD reported in descriptive European series of more than 150 affected children.

In adulthood, KD mostly affects young adults, with a mean age at diagnosis of 31 years (range 18–68) and a slight preponderance of males (male/female ratio 1.2) (38). The disease is rare and often misdiagnosed, with a median time to diagnosis of 13 days, cardiogenic shock in 5% of cases and a high rate of coronary artery aneurysms (19%) (38).

## GEOGRAPHIC VARIATIONS

Seasonal variations are coherent across Europe, but geographic variations seem discordant. In Germany, KD cases were widely distributed, with no correlation among incidence rates by state and state population density per square kilometer or land under farming (30). In the United Kingdom, more cases occurred in rural than urban areas (26). Conversely, in Portugal and in The Netherlands, the incidence of KD was higher in regions with a high population density and less agriculture-based economies (29, 35). In Catalonia, Spain, most of the cases were concentrated in the most densely populated areas around Barcelona, but a significant difference was observed for the 11.5% of patients living in rural areas vs. the expected 5% for the Catalan population according to the national census. Therefore, the authors concluded that KD was more prevalent in rural than urban areas of Catalonia (31). The incidence of KD was not related to population density in Italian regions, and the authors wondered if the higher incidence in some regions might reflect better reporting (19). The same discrepancies found in Asia (15, 49, 50) suggest that urbanization and industrialization are not major etiological factors in the occurrence of KD.

## ENVIRONMENTAL RISK FACTORS

An epidemiological study performed in two regions in Italy (Emilia and Romagna) found a negative correlation between temperature and number of KD cases but no correlation with precipitation or quality of air (33). Reduced onset during spring and summer seems to exclude a relation with pollen or fertilizers used in agriculture (33). The occurrence of two epidemic peaks in 2005 and 2013 in Romagna and a possible link with southerly winds and milder temperature in Emilia support the implication of an airborne infectious agent operating during winter months (33). Only 16% of Spanish patients had positive microbiological findings, but the causative agent was a respiratory virus in 35% of cases and pharyngeal Streptococcus group A in 29% (40). A tertiary pediatric center in Paris observed two peaks of hospital admission for KD between 2006 and 2020. The first peak in 2009 was concomitant with the H1N1 pandemic and the second in 2020 with the COVID-19 pandemic (51). These reports support the role of a non-exclusive upper respiratory-tract infection triggering KD. The association of social deprivation with increased incidence of KD in the United Kingdom could also support the causal role of infection but might also include other environmental determinants (24). To date, there is low evidence to incriminate exposure to other airborne agents such as pollutants or pollens (33).

## CONCLUSION

The annual incidence of KD in northern and western European countries is about 10–15 per 100,000 children under 5 years old and seems to be relatively stable over time and space. From the limited data available, the incidence seems to be similar in Eastern Europe. Demographic characteristics of KD in Europe are in line with those in other countries, with a higher incidence in children with Asian and possibly North African origins. Genetic predisposition is undeniable in this disease, but environmental factors appear to play a pivotal role. Several lines of evidence support that KD may have an infectious origin. Age of onset, self-limited course of the disease, coherent seasonality across Europe with more cases during winter and early spring, the occurrence of epidemic peaks during the H1N1 and COVID-19 pandemics, a possible link with southerly winds and elevated serum IgA level point to an airborne infectious agent operating during the winter months, with the host inducing a protective immune response. Overlapping features of KD with multisystem inflammatory disease in children described during the COVID-19 pandemic (52) reinforces the hypothesis of an airborne infectious trigger during KD. Studies of this newly described entity will help in understanding the pathogenic mechanisms involved in these two inflammatory diseases and possibly provide clues about host- and pathogen-dependent factors involved in the pathogenesis of KD.

## AUTHOR CONTRIBUTIONS

MP performed the review, drafted the manuscript, and agrees to be accountable for the content of the work.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.673554/full#supplementary-material

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# Overlapping Features in Kawasaki Disease-Related Arthritis and Systemic-Onset Juvenile Idiopathic Arthritis: A Nationwide Study in Japan

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**Background:** Arthritis may occur after the diagnosis of Kawasaki disease (KD). Most cases are self-limiting; however, some patients require prolonged treatment.

**Method:** To characterize KD-related arthritis, 14 patients who required arthritis treatment within 30 days after the diagnosis of KD were recruited from the 23rd KD survey in Japan. Twenty-six additional patients were included from our tertiary center and literature review cohorts.

**Results:** The estimated prevalence of KD-related arthritis in Japan was 48 per 100,000 KD patients. Patients with KD-related arthritis had an older age at onset (52 vs. 28 months, $P = 0.002$) and higher rate of intravenous immunoglobulin (IVIG) resistance in comparison to those without arthritis (86 vs. 17%, $P < 0.001$). Among 40 patients, 18 had arthritis in the acute phase KD (continued fever-onset type) and 22 did in the convalescent phase (interval fever-onset type). Both showed a similar rate of complete KD or IVIG response. Interval-type patients required biologics for arthritis control less frequently (5 vs. 39%, $P = 0.02$) and had a higher 2-year off-treatment rate (100 vs. 43%, $P = 0.009$) than continued-type ones. Interval-types showed lower serum ferritin and interleukin-18 levels than continued-types. When continued-types were

grouped according to whether or not they required biologics ($n = 7$ and $n = 11$, respectively), the former subgroup had higher ferritin and interleukin-18 levels ($P = 0.01$ and 0.02, respectively). A canonical discriminant analysis differentiated interval-type from continued-type with the combination of age, time to arthritis, and the ferritin and matrix metalloproteinase-3 levels.

**Conclusion:** Arthritis requiring treatment is a rare complication of KD. KD-associated arthritis includes interval-type (KD-reactive) and continued-type (true systemic-onset juvenile idiopathic arthritis [JIA] requiring biologics), and overlapping arthritis, suggesting the pathophysiological continuity of autoinflammation between KD and JIA.

**Keywords: Kawasaki disease, juvenile idiopathic arthiritis, arthritis, immunosuppressive therapy, biologics**

## INTRODUCTION

Kawasaki disease (KD) is an acute febrile vasculitis of unknown etiology that primarily occurs in infants and children (1). The systemic vasculitis affects small- and medium-sized arteries, predominantly the coronary arteries. The advent of intravenous immunoglobulin (IVIG) reduced the incidence of coronary artery abnormalities (CAAs) in patients from 20 to 25% to <5% (1). However, KD remains the leading cause of acquired cardiovascular disease in children in developed countries. The incidence of KD is highest in Asian populations, especially Japanese. No single genetic or pathogenic factor or environmental substance has been identified as a cause of KD. The presentation of KD starts with high fever, followed by the sequential development of cervical lymphadenopathy, non-purulent conjunctivitis, and polymorphic exanthema. On the other hand, extra-cardiac manifestations often occur, including aseptic meningitis, anterior uveitis, and arthritis of small joints during the course of illness (2). Although KD may arise from innate immune activation in genetically predisposed individuals (3–5), the pathogenesis and treatment of non-cardiovascular complications remain to be explained.

Arthritis is a complication of KD that occurred in up to one-third of patients during the pre-IVIG era (1). It is generally recognized as a self-limited and non-deforming condition that is not associated with the destruction of articular cartilage (6), whereas several cases have reportedly developed severe arthritis resulting in the diagnosis of systemic-onset juvenile idiopathic arthritis (SoJIA) (7). SoJIA is classified as a subtype of juvenile idiopathic arthritis (JIA); it accounts for 10–20% of all cases of JIA (8). SoJIA patients present with continuous fever and then develop arthritis during the febrile or afebrile phase after treatment. Although the infection and immunological mechanisms are implicated in the onset and progression of JIA, the etiology remains unclear.

The diagnosis of SoJIA or KD at the disease onset is challenging because of common principal features and due to the absence of biomarkers for each disease (7, 9–13). In the case of arthritis, a considerable observation period is needed to determine whether the main lesion is joint or vascular inflammation. Despite the different response to IVIG, KD and SoJIA share the pathophysiology of inflammation during the disease course. There is little information about the occurrence, clinical characteristics, or treatment outcomes of arthritis complicated with KD. KD-related arthritis is classified into the early-onset and late-onset types, which develop within 10 days after the onset of KD and more than 10 days after the onset of KD, respectively (6). Patients with KD-related arthritis are more frequently complicated by CAAs than those without it (14). Patients with SoJIA or KD are at risk of macrophage activation syndrome (MAS). The early control of KD-related arthritis may therefore reduce the life-threatening complications. However, the differentiation of IVIG-resistant KD and SoJIA, which is important for indicating second-line treatment with disease-modifying drugs including biologics, corticosteroids, and other immunomodulation therapy, remains a dilemma for pediatricians. The timing of the onset of KD-related arthritis; early- or late-onset, does not predict the outcomes of arthritis treatment or exclude the diagnosis of *true* SoJIA at the time of step-up treatment.

In order to facilitate the optimal treatment of KD-related arthritis after the early exclusion of SoJIA, the present study aimed to investigate the prevalence, clinical expression, and treatment of arthritis by a recent nationwide survey of KD in Japan. Because of the insufficient number of cases, we further studied the treatment response of KD and KD-related arthritis after collecting additional cases from other cohorts in our tertiary center and from a literature review. The mode of onset of arthritis effectively discriminated between KD-reactive arthritis from SoJIA, and left a small set of borderline cases.

## MATERIALS AND METHODS

### Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and the ethical guidelines for epidemiologic research of the Ministry of Health, Labor, and Welfare of Japan. Because of the retrospective study design, the requirement for written informed consent was waived. The study design was approved by the Institutional Review Board of Kyushu University (No. 28–94) and Jichi Medical University (No. 15–162).

## Diagnosis of KD

We used the diagnostic criteria of the Japanese KD Research Committee (14). Both complete and incomplete KD were included in the current study. Complete KD was defined by the presence of at least five of six principal symptoms, or the presence of four principal symptoms accompanied by CAAs. CAAs included dilations, aneurysms, stenosis, valvular heart disease, and myocardial infarction. Incomplete KD was diagnosed by the attending physicians. No KD-related arthritis was treated according to the same protocol that is used to treat JIA at the time of presentation.

## The Nationwide Survey of Patients Who Received the Treatment for Arthritis During the Course of KD

We conducted a nationwide study on patients who required active treatment for arthritis during the acute or subacute phase of KD (KD-related arthritis) as additional research of the 23rd nationwide survey of KD, which targeted patients diagnosed with KD from January 2013 to December 2014. Arthritis was determined by the presence of joint swelling, redness, and/or warmth, in addition to arthralgia. Otherwise, arthritis was confirmed by the signal change in the affected joints on magnetic resonance imaging. Patients with arthralgia alone, without other signs of arthritis were excluded from this study. The detailed methods of the survey have been described previously (15). Briefly, we sent questionnaires to all children's hospitals and institutions with $\geq$100 beds that had a pediatric department throughout Japan. The questionnaire included an initial questionnaire regarding the number of patients who required any treatment for arthritis within 30 days after the diagnosis of KD, as the definition of KD-related arthritis. The exclusion criteria were as follows: patients with dysmorphism or previously diagnosed disease. We then performed the secondary survey for each patient to determine the (1) background, (2) clinical characteristics and laboratory data at the time of the diagnosis of KD, (3) type of treatment of KD, (4) clinical and laboratory findings at the time of diagnosis of arthritis, (5) kind of treatment of arthritis and (6) outcomes. Arthritis treatments were classified into 5 categories: NSAIDs, corticosteroids, non-biologic immunosuppressants (methotrexate, cyclosporine A and tacrolimus), and biologic agents (infliximab, etanercept, canakinumab, and tocilizumab). Treatment outcomes were analyzed at 2 years after the diagnosis of KD.

## Chart and Literature Reviews of KD-Related Arthritis

In addition to the nationwide survey, we conducted another cohort analysis by chart and literature reviews as follows, because many of these rare cases did not include detailed information. We retrospectively investigated all patients with KD-related arthritis, as defined above, who received treatment at Kyushu University Hospital from 2007 to 2019. We further included all reported cases of KD-related arthritis by a review of the literature. Using the terms of "KD" and "arthritis," we searched the PubMed database in June 2020 to identify publications describing these cases. The demographics, clinical characteristics, and all treatments were then extracted.

## Statistical Analyses

We used a chi-squared test to compare the proportions of categorical variables and the Mann-Whitney *U* test to compare the medians of continuous variables between the two groups. Bonferroni adjustment was used for multiple comparisons. For the analysis of long-term outcome, we included 19 patients only from the nationwide survey and our institution cohorts in order to dwarf possible difference in patient characteristics beyond statistical adjustments. A canonical discriminant analysis was performed to construct a prediction model, as we described previously (16). We included the months of age, days of illness at the onset of arthritis, and the serum ferritin and metalloproteinase-3 levels in a discriminant analysis. The Shapiro-Wilks test confirmed the normal distribution of these 4 variables. *P* values of $<0.05$ were considered to indicate statistical significance. The JMP Pro software program (ver. 14.2.0.; SAS Institute, Cary, NC, USA) was used for all of the statistical analyses.

# RESULTS

## KD-Related Arthritis in the 23rd Nationwide Survey of Japan

A total of 1,456 of the 1,943 eligible institutions replied to the initial questionnaire about KD-related arthritis in the 23rd nationwide KD survey (response rate, 74.9%), in which 31,679 patients received a diagnosis of KD in 950 institutions (**Supplementary Figure 1**). We excluded 2,595 patients who had $<$3 principal symptoms of KD or who improved spontaneously. Among 29,084 KD patients, 15 patients developed arthritis that required additional treatment. One patient was excluded because of confirmation of a $>$6 month-interval between the onset of arthritis and KD; thus, the pathophysiological association was undetermined. Fourteen patients with KD-related arthritis were finally identified (**Figure 1**). No patients had any previous history of arthritis at the onset of KD. The estimated prevalence of KD-related arthritis was 48 (95% confidence interval: 26–81) per 100,000 KD patients.

## KD-Related Arthritis in the Other Cohort

Nine patients with KD-related arthritis who were managed in our institution in this decade were identified. One was excluded as the interval between the onset of arthritis and KD was $>$6 months. The literature review identified 18 cases with KD and then arthritis. We also identified other 24 patients with KD-related arthritis but excluded them because of the onset of arthritis was very late ($>$6 months; $n = 6$) or due to a lack of detailed clinical information ($n = 18$). A total of 26 patients were included in the other cohort (**Figure 1**). No cases in this cohort overlapped with those in the data of the 23rd nationwide survey.

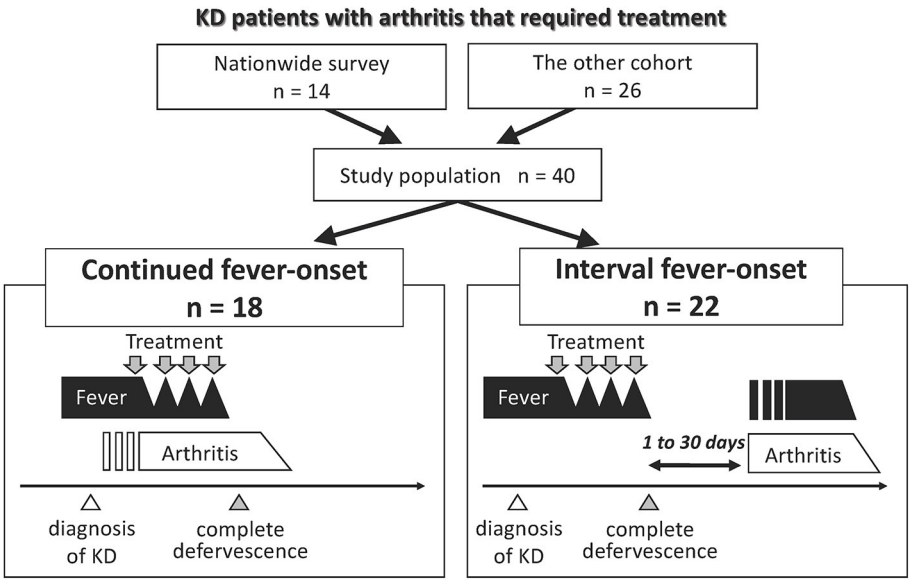


**FIGURE 1 |** Group classification of patients with KD-related arthritis. Fourteen and 26 patients who suffered from arthritis that required treatment until 30 days following the diagnosis of KD were extracted from the 23rd nationwide survey in Japan and the additional cohorts, respectively. We combined two cohorts and classified the patients into two groups according to the mode of onset of fever and arthritis. Open triangle represents the time of the diagnosis of KD. Gray triangle represents the time point of complete defervescence. Gray arrow indicates the point when patients received treatment for KD or arthritis.

## Comparison Between KD Patients With and Without Arthritis

The demographics and clinical characteristics of KD were compared among KD patients who did not develop arthritis that required treatment in the nationwide survey (cohort 1), patients with KD and arthritis that required treatment in the nationwide survey (cohort 2), and the other cohort (cohort 3) (**Table 1**). There was no significant difference in sex, days of illness at the time of the diagnosis of KD, or type of KD among the three cohorts. The age at the onset of KD in cohorts 2 and 3 was older than that in cohort 1 (median, 52 and 45 months of age, respectively, vs. 28 months of age, $P = 0.002$ and 0.008, respectively). The rates of resistance to initial IVIG in cohorts 2 and 3 were much higher than those seen in KD patients without arthritis that required treatment (86 and 69%, respectively, vs. 17%, $P \leq 0.001$ and $<0.001$, respectively). The rate at which additional treatments for KD were required did not differ between cohorts 2 and 3.

## Arthritis Developing in the Acute or Convalescent Phases of KD

There was no death due to CAAs or macrophage activation syndrome (MAS). We focused on two dissimilar courses of KD-related arthritis: the development of arthritis from the start of KD treatment to 24 h after complete defervescence was classified as continued fever-onset type, while the development of arthritis during the period from 1 to 30 days after complete defervescence was defined as the interval fever-onset type. According to these definitions, 18 and 22 patients were classified into the continued fever-onset and interval fever-onset groups, respectively. Complete defervescence was defined as a maximum body temperature of $<37.5^{\circ}$C for 24 consecutive hours. The clinical laboratory findings and treatment outcomes of patients with the continued fever and interval fever types are summarized in **Tables 2**, **3**, respectively. Despite the high rate of resistance to initial IVIG in both groups, the additional treatment of KD was only effective for 8 of 18 (44%) patients with the continued fever type, but was effective for all 22 patients with the interval fever type (**Table 4**). Continued fever-onset patients developed MAS more frequently (57 vs. 7%, $P = 0.03$) during the treatment course of arthritis. The serum matrix metalloproteinase-3 (MMP-3) levels in continued fever-onset cases were lower than those in the interval fever-onset cases (median, 45 vs. 160 ng/mL, $P = 0.02$). On the other hand, the serum ferritin level in the continued fever type was higher than that seen in the interval fever type (median, 3,163 ng/mL vs. 85 ng/mL, $P < 0.001$) The interleukin-18 (IL-18) levels in continued fever-onset patients were higher than those in interval fever-onset patients; however, the difference did not reach the statistical significance (51,500 vs. 711 pg/mL, $P = 0.051$). There was no difference in the numbers, locations, or symmetricity of the affected joints, or in autoantibody positivity. In both groups arthritis frequently involved the large joints of the lower limbs (knee 86 vs. 88%, hip 27 vs. 44%, and ankle 55 vs. 31%) and the small joints of upper limbs (wrist 60 vs. 43% and finger 42 vs. 50%). No patients were positive for rheumatoid factor or had an antinuclear antibody titer of $>1:80$. For the control of arthritis, 36% of the interval fever-type patients required NSAIDs alone ($P = 0.03$). In contrast, continued fever-onset patients required biologic agents more frequently in comparison to interval fever-onset patients (39 vs. 5%, $P = 0.02$). Focusing on the patients from the two cohorts

**TABLE 1 |** Demographic characteristics of KD patients with and without arthritis that required treatment.

| | 1 | 2 | 3 | *P* | | |
|---|---|---|---|---|---|---|
| | **KD without arthritis that required treatment, nationwide survey** | **KD with arthritis that required treatment, nationwide survey** | **KD with arthritis that required treatment, additional cohort** | **1 vs. 2** | **1 vs. 3** | **2 vs. 3** |
| Number of patients | 29,069 | 14 | 26 | | | |
| Months of age | 28, 0–279 | 52, 20–87 | 45, 3–164 | 0.002 | 0.008 | 0.23 |
| Male | 16,658 (57%) | 7 (50%) | 13 (50%) | 1.0 | 0.55 | 1.0 |
| Days of illness at diagnosis of KD | 5, 1–27 | 5, 3–9 | 5, 3–17 | 0.75 | 0.47 | 1.0 |
| Complete KD | 24,560 (85%) | 11 (79%) | 18 (69%) | 1.0 | 0.10 | 0.72 |
| 1st IVIG resistance | 4,986 (17%) | 12 (86%) | 18 (69%) | <0.001 | <0.001 | 0.45 |
| **Additional treatment** | | | | | | |
| Repeated IVIG | 5,573 (19%) | 13 (93%) | 19 (73%) | <0.001 | <0.001 | 0.22 |
| Infliximab | 295 (1%) | 2 (14%) | 5 (19%) | 0.026 | <0.001 | 1.0 |
| Plasma exchange | 149 (0.5%) | 1 (7%) | 1 (4%) | 0.21 | 0.13 | 1.0 |

*Data are shown as the number (%) or median and range. Bonferroni adjustment was used for multiple comparisons. KD, Kawasaki disease; JIA, juvenile idiopathic arthritis; IVIG, intravenous immunoglobulin.*

other than the literature review cohort, 4 of 7 (57%) continued fever-onset patients were treatment-dependent at 2 years after the onset of arthritis, but all of the 12 interval fever-onset patients were weaned from therapy ($P = 0.009$).

## Difference Between Continued Fever-Onset Patients Who Required Biologics and Those Who Did Not

The above data indicated that continued fever-onset patients required long-term aggressive treatment for arthritis, whereas the arthritis of the interval fever-onset patients was relatively mild and transient. We then focused on the clinical difference between the continued fever-onset patients who required biologics (continued fever-onset/biologic, $n = 11$) and those recovered without biologics (continued fever-onset/non-biologic, $n = 7$) (**Table 5**). There was no significant difference in age, sex, duration until the diagnosis of KD and arthritis, type of KD, or the frequency of CAAs and MAS. On the other hand, the serum levels of ferritin and IL-18 were significantly higher in the continued fever-onset/biologic patients (median, 718 ng/mL vs. 12,330 ng/mL, $P = 0.01$; 997 pg/mL vs. 136,000 pg/mL, $P = 0.02$). All four patients in the continued fever-onset/biologics group were still receiving treatment for arthritis at the 2-year-follow-up examination, although all three patients in the continued fever-onset/non-biologic group had become medication-free ($P = 0.03$) (**Figure 2**).

## Three Types of KD-Arthritis Discriminated by the Prediction Model

Among the five variables, including age, serum levels of ferritin, IL-18, and MMP-3 level, and days of illness at the onset of arthritis, only ferritin showed a significant difference among interval fever-onset, continued fever-onset/non-biologic, and continued fever-onset/biologic patients (**Figures 3A,B** and **Supplementary Figure 2**). We then focused on generating a prediction model that would be useful for facilitating an early diagnosis and intervention based on the abovementioned variables, with the exception of IL-18 due to the limited sample size. A canonical discriminant analysis effectively distinguished patients in the continued fever-onset/biologic, continued fever-onset/non-biologic, and interval fever-onset groups (Wilks' lambda 0.11, $P \leq 0.001$) (**Figure 3C**). We applied log transformation to each variable for the transformation of non-Gaussian data into Gaussian data. The normality of the data for each variable was estimated by the Shapiro-Wilks test ($P =$ 0.70, 0.25, 0.08, and 0.87, respectively). The scoring coefficients in the canonical plot were as follows: Canonical 1 = 0.90 Log [serum ferritin level, ng/mL]−0.18 Log [days of illness at the onset of arthritis]−0.19 Log [serum MMP-3 level, ng/mL] + 0.19 Log [months of age], Canonical 2 = 0.36 Log [serum ferritin level, ng/mL] + 0.91 Log [days of illness at the onset of arthritis]−0.02 Log [serum MMP-3 level, ng/mL]−0.56 Log [months of age]. However, this graph revealed that one interval fever-onset patient with complete KD (#40) had the properties of a continued fever-onset/biologic patient. This patient developed arthritis 8 days after the initial defervescence of KD, but showed a high serum level of ferritin at the time of the onset of arthritis. Unfortunately, no data were available for MMP-3, ferritin, or the IL-18 level in one interval fever-onset patient with methotrexate-dependent arthritis (#25) (**Figure 2**). The classification of 40 KD-arthritis patients into the early-onset and late-onset types did not make distinction between true SoJIA (after 2 years of treatment) and reactive arthritis (treatment-free at 2 years) (data not shown).

# DISCUSSION

The first nationwide survey of KD-related arthritis revealed that the prevalence of KD-related arthritis was 48 per 100,000 KD patients in Japan. KD patients with arthritis that required treatment showed higher resistance to initial IVIG than those

**TABLE 2 |** Detailed information of the continued fever-onset (*Co.*) patients.

| # | Gr. | Age, y.o | Sex | Type of KD | CAAs | Days of illness | | Laboratory data after the development of arthritis | | | Treatment for KD | | Treatment for arthritis | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dx. of KD | Onset of arthritis | MMP3, ng/mL | Ferritin, ng/mL | IL-18, pg/mL | Course | Effect | Acute phase | After 2 years | |
| 1 | *Co* | <0.5 | M | I | D | 6 | 15, F | na | 653 | na | IVIG→IVIG→IVIG→CS | Yes | CS | na | (17) |
| 2 | *Co* | 0.5–2 | M | C | D | na | na, F | na | Na | na | IVIG→IVIG | No | NSAIDs, CS, MTX | na | (18) |
| 3 | *Co* | 0.5–2 | M | I | D | na | na, F | na | Na | na | IVIG→IVIG/CS | Yes | CS, MTX, ETN, CAN, TCZ | na | 9 |
| 4 | *Co* | 2–4 | M | C | na | 4 | 11, F | na | Na | na | IVIG→IVIG/CS | Yes | NSAIDs, CS | na | 6 |
| 5 | *Co* | 2–4 | M | C | na | 6 | 4, F | na | Na | na | IVIG→IVIG | Yes | NSAIDs, CS | na | 6 |
| 6 | *Co* | 2–4 | M | I | No | 9 | 14, FM | 15 | 64,859 | 51,500 | IVIG→IVIG→IFX→IVIG | No | NSAIDs, CS, HDMP, CyA, TCZ | na | NWS |
| 7 | *Co* | 2–4 | F | C | No | 3 | 4, F | 116 | 495 | 1,128 | IVIG→IVIG→CyA | Yes | CyA | na | 11 |
| 8 | *Co* | 4–6 | M | I | PB | 9 | 12 | na | 19,740 | 132,000 | IVIG/CS | Yes | CS | na | 12 |
| 9 | *Co* | 4–6 | M | I | na | 17 | 8 | na | Na | na | IVIG | Yes | NSAIDs | na | 6 |
| 10 | *Co* | 4–6 | F | C | No | 6 | 6, F | 23 | 313 | 865 | IVIG→IVIG→IFX→IVIG | Yes | NSAIDs, CS | No | KU |
| 11 | *Co* | 4–6 | F | C | No | 6 | 6, FM | 32 | 7,204 | 136,000 | IVIG/CS→IVIG→IVIG→IVIG | No | NSAIDs, CS, HDMP, CyA, TCZ, VP-16, PE | Yes | NWS |
| 12 | *Co* | 6–8 | F | C | D | na | na, F | na | Na | na | IVIG→IVIG | No | CS, MTX, IFX | na | (18) |
| 13 | *Co* | 6–8 | F | C | No | 5 | na, F | 29 | 2,700 | 17,600 | IVIG→IVIG | No | CS, HDMP | na | NWS |
| 14 | *Co* | 6–8 | F | C | No | 9 | 16, FM | 24 | 12,330 | 68,000 | IVIG→IVIG→HDMP | No | CS, CyA, TCZ | Yes | NWS |
| 15 | *Co* | 6–8 | F | I | No | 5 | 31, F | 81 | 3,626 | 202,000 | IVIG→IVIG→PE | No | CS, CyA, TCZ | Yes | NWS |
| 16 | *Co* | 6–8 | F | I | No | 13 | 12, F | 190 | 15,600 | 145,000 | IVIG→IVIG→UTI | No | NSAIDs, MTX, TCZ | Yes | KU |
| 17 | *Co* | >8 | M | C | No | 3 | 8, F | 135 | 752 | 586 | IVIG→IVIG→IFX→PE | No | NSAIDs, MTX, CyA | No | KU |
| 18 | *Co* | >8 | F | C | No | 4 | 6, FM | 58 | 718 | 495 | IVIG→HDMP | No | NSAIDs, CS, HDMP, TAC | No | KU |

*KD, Kawasaki disease; C, complete KD; I, incomplete KD; CAAs, coronary artery abnormalities; D, dilation; PB, perivascular brightness; Gr., group; Dx., diagnosis; F, fever; FM, fever and macrophage activation syndrome; MMP3, matrix metalloproteinase-3; IL-18, Interleukin-18; IVIG, intravenous immunoglobulin; CS, corticosteroids; IFX, infliximab; CyA, cyclosporine A; HDMP, high-dose methylprednisolone; UTI, ulinastatin; PE, plasma exchange; NSAIDs, non-steroidal anti-inflammatory drugs; MTX, methotrexate; ETN, etanercept; CAN, canakinumab; TCZ, tocilizumab; ANR, anakinra; VP-16, etoposide; TAC, tacrolimus; NWS, Nationwide survey; KU, Kyushu University; na, not available.*

**TABLE 3 |** Detailed information of the interval fever-onset (*Int.*) patients.

| # | Gr. | Age, y.o | Sex | Type of KD | CAAs | Days of illness | | Laboratory data after the development of arthritis | | | Treatment for KD | | Treatment for arthritis | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Dx. Of KD | Onset of arthritis | MMP3, ng/mL | Ferritin, ng/mL | IL-18, pg/mL | Course | Effect | Acute phase | After 2 years | |
| 19 | *Int* | 0.5–2 | M | C | No | 3 | 18, F | 9 | 29 | 1,140 | IVIG→IVIG/HDMP→CyA | Yes | NSAIDs, CS, CyA | No | NWS |
| 20 | *Int* | 0.5–2 | M | C | D | 3 | 28, F | 195 | 258 | 990 | IVIG→IFX→IVIG→IFX | Yes | NSAIDs, CS, MTX | No | KU |
| 21 | *Int* | 0.5–2 | F | C | D | 4 | 14, F | 216 | 99 | na | IVIG | Yes | NSAIDs | No | KU |
| 22 | *Int* | 0.5–2 | F | C | na | na | na | na | Na | na | IVIG→IVIG | Yes | NSAIDs, CS | na | (19) |
| 23 | *Int* | 2–4 | M | C | No | 4 | 9, F | 83 | 97 | na | IVIG→IVIG/CS | Yes | NSAIDs, CS, MTX, CyA | No | NWS |
| 24 | *Int* | 2–4 | M | C | No | 7 | 21, F | 59 | 13 | 432 | IVIG→IVIG/HDMP→CyA | Yes | NSAIDs, CS, CyA | No | NWS |
| 25 | *Int* | 2–4 | M | I | No | 16 | Na | na | Na | na | IVIG→IVIG→HDMP | Yes | CS, MTX | Yes | 7 |
| 26 | *Int* | 2–4 | F | C | No | 4 | 6 | 181 | 67 | na | IVIG | Yes | NSAIDs | No | KU |
| 27 | *Int* | 2–4 | F | C | No | 5 | 26 | na | 71 | na | IVIG/CS | Yes | NSAIDs, CS | No | NWS |
| 28 | *Int* | 2–4 | F | C | No | 5 | 15, F | 187 | 278 | na | IVIG→IVIG→HDMP | Yes | NSAIDs, CS | No | NWS |
| 29 | *Int* | 2–4 | F | C | A | 5 | 30, F | 184 | 46 | 166 | IVIG→IVIG→HDMP→IFX | Yes | NSAIDs, MTX | No | KU |
| 30 | *Int* | 2–4 | F | C | No | na | 17, F | 279 | Na | na | IVIG→IVIG | Yes | NSAIDs | na | (20) |
| 31 | *Int* | 2–4 | F | C | na | na | na | na | Na | na | IVIG | Yes | NSAIDs | na | (19) |
| 32 | *Int* | 4–6 | M | C | D | 4 | 10, F | na | Na | na | IVIG | Yes | NSAIDs | na | (21) |
| 33 | *Int* | 4–6 | M | C | D | 4 | 21, F | 103 | Na | na | IVIG→IVIG→IVIG/CS | Yes | CS | No | NWS |
| 34 | *Int* | 4–6 | M | C | No | 4 | 28 | 139 | Na | na | IVIG→IVIG | Yes | NSAIDs, CS | No | NWS |
| 35 | *Int* | 4–6 | M | C | No | 5 | 23, F | 244 | 72 | na | IVIG→IVIG/CS | Yes | NSAIDs | No | NWS |
| 36 | *Int* | 4–6 | M | I | No | 7 | na, FM | na | 8,506 | na | IVIG→IVIG→IFX | Yes | CS, ANR | No | 7 |
| 37 | *Int* | 4–6 | F | C | No | 5 | 14, F | 118 | Na | na | IVIG | Yes | NSAIDs | na | (20) |
| 38 | *Int* | 6–8 | F | I | D, T | 15 | 29, F | na | Na | na | IVIG→IVIG→HDMP | Yes | NSAIDs, CS | na | (22) |
| 39 | *Int* | 6–8 | M | C | na | na | na | na | Na | na | IVIG→IVIG | Yes | NSAIDs | na | (19) |
| 40 | *Int* | 6–8 | F | I | No | 7 | 20 | 39 | 3,242 | na | IVIG→IVIG/CS→IFX | Yes | na | na | NWS |

*KD, Kawasaki disease; C, complete KD; I, incomplete KD; CAAs, coronary artery abnormalities; A, aneurysm; D, dilation; T, thrombus formation; Gr.,group; Dx., diagnosis; F, fever; FM, fever and macrophage activation syndrome; MMP3, matrix metalloproteinase-3; IL-18, Interleukin-18; IVIG, intravenous immunoglobulin; HDMP, high-dose methylprednisolone; CyA, cyclosporine A; IFX, infliximab; NSAIDs, non-steroidal anti-inflammatory drugs; CS, corticosteroids; NWS, Nationwide survey; KU, Kyushu University; na, not available.*

**TABLE 4 |** Clinical characteristics of registered patients with KD and arthritis that required treatment.

| | Continued fever-onset | Interval fever-onset | *P* |
|---|---|---|---|
| Number of patients | 18 | 22 | |
| Months of age | 60, 3–164 | 46, 9–86 | 0.14 |
| Male | 9/18 (50%) | 11/22 (50%) | 1.0 |
| Days of illness at the diagnosis of KD | 6, 3–17 | 5, 3–16 | 0.24 |
| Days of illness at the onset of arthritis | 10, 4–31 | 20, 6–30 | 0.003 |
| Complete KD | 11/18 (61%) | 18/22 (82%) | 0.17 |
| **Complications** | | | |
| Coronary artery abnormalities | 5/15 (33%) | 6/19 (32%) | 1.0 |
| Macrophage activation syndrome | 4/7 (57%) | 1/14, (7%) | 0.03 |
| **Treatment response** | | | |
| Response to single IVIG | 2/18 (11%) | 9/22 (41%) | 0.07 |
| Response to additional KD therapy[a] | 8/18 (44%) | 22/22 (100%) | <0.001 |
| **Laboratory data at onset of arthritis** | | | |
| Rheumatoid factor, %positive | 0/9 (0%) | 0/18 (0%) | - |
| Matrix metalloproteinase-3, ng/mL | 45, 15–190 | 160, 9–279 | 0.02 |
| Ferritin, ng/mL | 3,163, 313–64,859 | 85, 13–8,506 | <0.001 |
| Interleukin-18, pg/mL | 51,500, 495–202,000 | 711, 166–1,140 | 0.05 |
| **Treatment for arthritis** | | | |
| NSAIDs alone | 1/18 (6%) | 8/22 (36%) | 0.03 |
| Corticosteroids | 15/18 (83%) | 12/21 (57%) | 0.10 |
| Non-biologic immunosuppressants[b] | 11/18 (61%) | 6/21 (29%) | 0.06 |
| Biologic agents[c] | 7/18 (39%) | 1/21 (5%) | 0.02 |
| Except for infliximab | 6/18 (33%) | 1/21 (5%) | 0.04 |
| Off treatment at 2 years' follow-up | 3/7 (43%) | 12/12 (100%) | 0.009 |

*Data are shown as the number (%) or median and range.*

[a] *Additional KD therapy includes conventional or high-dose corticosteroids, infliximab, plasma exchange transfusion, and ulinastatin other than IVIG.*

[b] *Non-biologic immunosuppressants include methotrexate and cyclosporine A.*

[c] *Biologic agents include infliximab, etanercept, tocilizumab, and canakinumab. KD, Kawasaki disease; IVIG, intravenous immunoglobulin; NSAIDs, non-steroidal anti-inflammatory drugs.*

without arthritis. KD-related arthritis had two dissimilar patterns of expression: the refractory type, which occurred in the acute febrile phase; and the curable type, which occurred in the convalescent phase. This indicates the different pathophysiology associated with the joint inflammation of the two types. Furthermore, among the refractory type, patients who finally required biologics had high levels of serum ferritin and IL-18 in the acute phase and often required aggressive treatment, whereas those who improved without biologics had only moderate levels of ferritin and IL-18. The serum levels of ferritin and IL-18 at the onset of arthritis may be a good predictive biomarker to distinguish among these three types of KD-related arthritis. The discriminant model using the clinical variables at the onset of arthritis may help to more accurately classify the borderline disease between cases of KD-related arthritis and SoJIA.

The characteristics of interval fever-onset arthritis also resembled those of reactive arthritis (ReA). ReA occurs at 1–4 weeks after bacterial infection or inflammatory bowel disease, predominantly affecting the large joints of the lower extremities and occasionally affecting the wrists and fingers (23). No autoantibodies are found to be associated with the development of ReA. It usually resolves within 6 months without recurrence. In this context, interval fever-onset arthritis could be defined as ReA after KD, or KD-reactive arthritis.

High IVIG resistance was a hallmark of KD-related arthritis, including the interval fever-onset type. Considering that prolonged systemic inflammation may trigger arthritis, KD and ReA may be categorized into the disease spectrum of auto-inflammation. Recently, innate immunity has been recognized as a key contributor to the development of both KD and ReA. In mouse models (4), nucleotide-binding oligomerization domain-containing protein 1 ligand induced KD-like coronary vasculitis. It has also been reported that persistent gastrointestinal or urogenital infection is the source of causative pathogen-associated molecular patterns and leads to the development of ReA in association with a toll-like receptor 2 pathway (24). In fact, several pathogens, such as *Yersinia*, *Chlamydia*, and *Mycoplasma*, have been reported to be associated with the development of both KD and ReA (5, 25, 26). *Yersinia*-induced reactive arthritis is also related to the development of vasculitis (27). Understanding the shared mechanism of autoinflammation in KD and ReA may elucidate the pathogenesis of these diseases.

With the exception of one patient (Patient #8), patients with continued fever-onset arthritis had sustained fever at the

**TABLE 5 |** Clinical characteristics of the continued fever-onset patients who required biologics for arthritis control and those who did not.

| | Biologics not required | Biologics required | *P* |
|---|---|---|---|
| Number of patients | 11 | 7 | |
| Months of age | 60, 3–164 | 72, 10–87 | 0.59 |
| Male | 7/11 (63%) | 2/7 (29%) | 0.33 |
| Days of illness at the diagnosis of KD | 6, 3–17 | 9, 5–13 | 0.13 |
| Days of illness at the onset of arthritis | 8, 4–15 | 14, 6–31 | 0.06 |
| Complete KD | 8/11 (73%) | 3/7 (43%) | 0.33 |
| **Complications** | | | |
| Coronary artery abnormalities | 3/9 (33%) | 2/9 (22%) | 1.0 |
| Macrophage activation syndrome | 1/4 (25%) | 3/3 (100%) | 0.14 |
| **Treatment response** | | | |
| Single IVIG | 2/11 (18%) | 0/7 (0%) | 0.50 |
| Additional KD therapy[a] | 7/11 (63%) | 1/7 (14%) | 0.66 |
| **Laboratory data at onset of arthritis** | | | |
| Matrix metalloproteinase-3, ng/mL | 58, 23–135 | 32, 15–190 | 0.52 |
| Ferritin, ng/mL | 718, 313–19,740 | 12,330, 3,626–64,859 | 0.01 |
| Interleukin-18, pg/mL | 997, 495–132,000 | 136,000, 500–202,000 | 0.02 |
| **Treatment for arthritis** | | | |
| NSAIDs alone | 1/11 (9%) | 0/7 (0%) | 1.0 |
| Corticosteroids | 9/1 (82%) | 6/7 (86%) | 1.0 |
| Non-biologic immunosuppressants[b] | 4/11 (36%) | 7/7 (100%) | 0.01 |
| Off treatment at 2 years' follow-up | 3/3 (100%) | 0/4 (0%) | 0.03 |

*Data are shown as the number (%) or median and range.*

[a]*Additional KD therapy includes conventional or high-dose corticosteroids, infliximab, plasma exchange transfusion, and ulinastatin other than IVIG.*

[b]*Non-biologic immunosuppressants include methotrexate and cyclosporine A. KD, Kawasaki disease; IVIG, intravenous immunoglobulin; NSAIDs, non-steroidal anti-inflammatory drugs.*

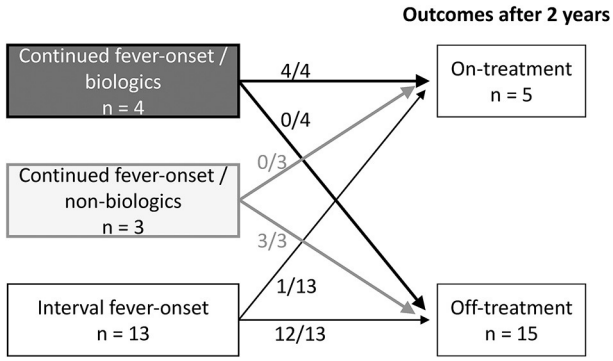


**FIGURE 2 |** The treatment outcomes of three group-patients with KD-related arthritis. Information of the treatment outcomes of KD-related arthritis was available for 4 of 7 continued fever-onset patients /biologics, 3 of 11 continued fever-onset /non-biologics patients, and 13 of 22 interval fever-onset patients. All four continued fever-onset patients with arthritis that required biologic therapy and one interval fever-onset patient were receiving treatment at 2 years after the onset of disease. On the other hand, all three continued fever-onset patients who were managed without biologics and 12 of the 13 interval fever-onset patients were off treatment.

onset of arthritis during the treatment of KD. Continued fever-onset/biologic patients showed high serum levels of ferritin and IL-18 and frequently became chronic, which were characteristics of SoJIA (28, 29). There have been several case reports of SoJIA after receiving the diagnosis of KD (7, 10, 13). The early diagnosis of SoJIA is difficult because it starts with high fever, skin rash, and lymphadenopathy, which is not differentiated from KD, without apparent arthritis (8, 30). In this setting, the majority of continued fever-onset/biologic patients might otherwise be "true SoJIA," although the first presentation fulfilled the diagnostic criteria of KD. CAA is one of the common and specific complications of KD, whereas some patients finally diagnosed as SoJIA also developed CAAs (Patients #1–3, 8, and 12) (**Tables 2**, **3**). We therefore compared the characteristics of CAAs developed in continued-fever onset and interval-onset fever group and found that there was no significant difference in the characteristics of CAAs between these groups. Patients in the continued fever-onset/non-biologic group also developed arthritis during the acute febrile phase. However, they showed moderate levels of ferritin and IL-18, and had a transient and curable course, similar to ReA. These patients might have had ReA with more excessive inflammation than typical ReA or the overlapping condition of SoJIA and KD-related ReA. The present study showed that the serum levels of ferritin and IL-18 can be useful to objectively predict the treatment response of arthritis during the course of KD. Our discriminant model may effectively differentiate the ReA type (interval fever-onset), SoJIA mimicking-KD type (continued fever-onset/biologic), and the intermediate type (continued fever-onset/non-biologic). It

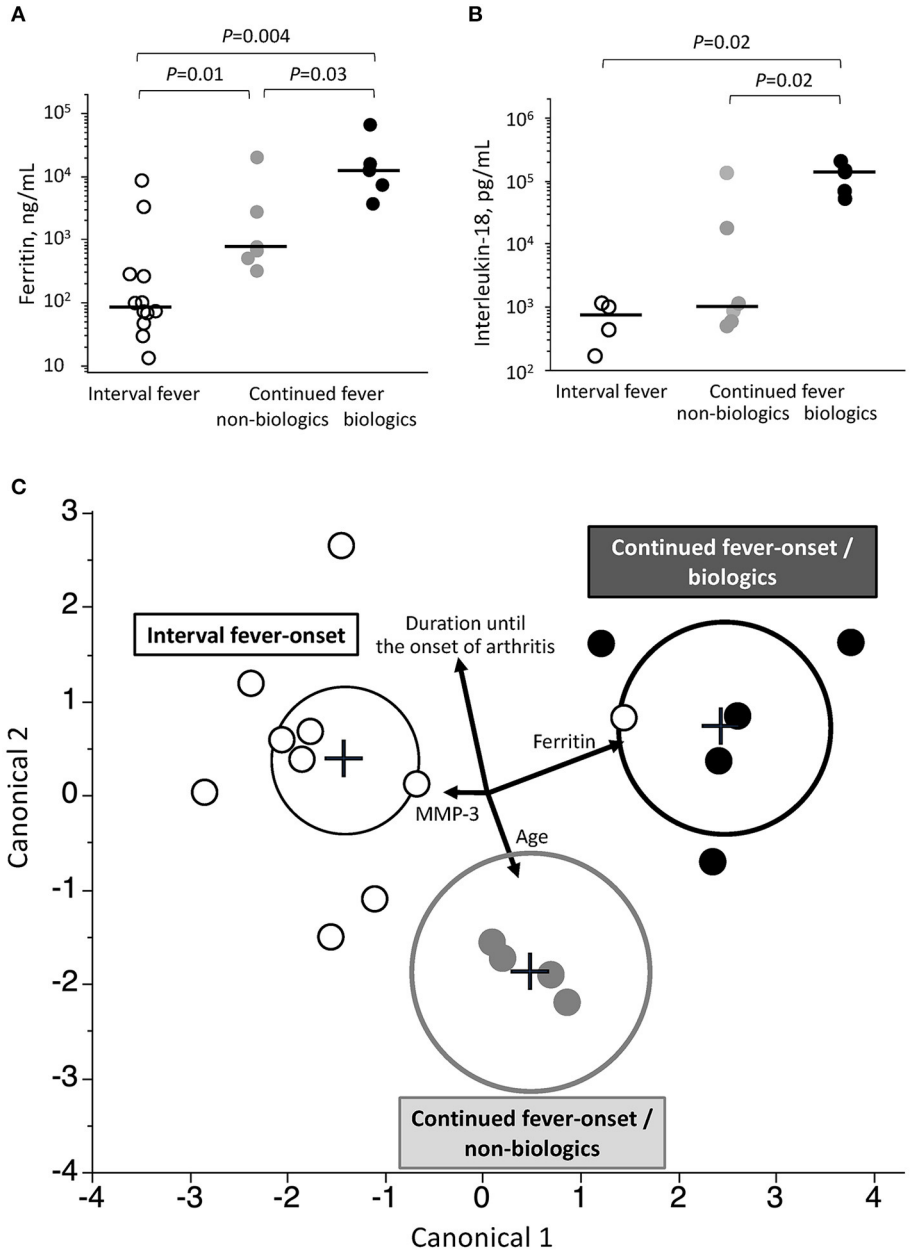


**FIGURE 3 |** Associated clinical variables among the three groups of patients with KD-related arthritis. **(A,B)** Comparison of serum levels of ferritin **(A)** and interleukin-18 **(B)** at the onset of arthritis among patients in the continued fever-onset/biologic, continued fever-onset/non-biologic, and interval fever-onset groups. Black bars represent the median values. **(C)** A prediction model to predict the three types of the KD-related arthritis based on a canonical discriminant analysis. Each plot is determined using the combination of 4 clinical covariates at the onset of arthritis. Vectors indicate the direction and magnitude of each element. Black, gray, and white plots represent patients in the continued fever-onset/biologic, continued fever-onset/non-biologic, and interval fever-onset groups, respectively. The black, gray, and dotted circles in **(B)** denote the 95% confidence ellipse in the continued fever/biologic, continued fever-onset/non-biologic, and interval fever-onset types, respectively.

indicates that the combination of clinical variables at the onset of arthritis may be useful for pediatricians to select treatment and predict the joint prognosis.

The present study should be carefully interpreted as follows. First, the prevalence of KD-related arthritis was lower than those of KD-associated arthritis in reported in previous single-center studies in the IVIG era (2–13% of KD patients) (6, 19, 31, 32). This is explained by the recruitment of subjects: patients who needed any treatment for arthritis, but not the mild or self-limited form of arthritis, thus the small number of cases with KD-related arthritis might not typify the disease entity. Second, this study was based on the nationwide study of KD, although a part of patients, especially continued-fever onset/biologic patients, might have SoJIA as discussed above. Differentiation between KD

and SoJIA is often difficult because both diseases are diagnosed on the basis of clinical symptoms. Our results emphasize the importance of considering SoJIA in patient with refractory KD even if they fulfilled the diagnostic criteria of KD. Third, 2-year outcome was available only for 19 patients among 22 from the nationwide survey and our institution cohorts. It is crucial to test whether the long-term outcome in the present study can be corroborated in other studies, thus prospective registration of KD patients targeting at long-term prognosis of arthritis and related symptoms are needed for the complete cure of KD.

## CONCLUSIONS

We conducted the first nationwide survey of KD patients who developed arthritis that required treatment. These patients were classified into three groups: (1) SoJIA mimicking KD, (2) KD-related ReA, and (3) arthritis with overlapping features between SoJIA and ReA. The serum levels of ferritin and IL-18 or the combination of clinical covariates at the onset of arthritis may help us to predict the treatment response and prognosis of arthritis. Although continued fever-onset patients were more likely to be treatment-dependent at 2 years after the onset of arthritis as compared to interval fever-onset patients in the present analysis, further studies are needed for the conclusion validity on the long-term prognosis. These findings, although preliminary, are potentially pivotal for risk stratification and treatment selection for patients who develop arthritis during the course of KD.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Kyushu University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

HK contributed to the protocol development, performed the statistical analyses, interpreted the data, and drafted the initial manuscript. EN contributed to the protocol development, interpreted the data, and drafted the initial manuscript. HT contributed to the protocol development and critically revised the manuscript. MI and HN assisted in the protocol development and reviewed the manuscript. SHo (a Master of Science in Epidemiology) contributed to the statistical analysis and reviewed the manuscript. HM, NN, TN, TI, TA, SHa, LL, YT, JK, OK, UK, ST, AS, and SHi collected the data and reviewed the manuscript. MY, NM, and YN managed the data collection and reviewed the manuscript. TH conceptualized and designed the study and reviewed the manuscript. SO supervised the study and critically revised the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.597458/full#supplementary-material

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