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Original Article

Respiratory viral infections and Kawasaki disease: A molecular epidemiological analysis

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Abstract *Background/Purpose:* Recent large-scale epidemiological studies have revealed significant temporal associations between certain viral infections and the subsequent development of Kawasaki disease (KD). Despite these associations, definitive laboratory evidence linking acute or recent viral infections to KD cases remains elusive. The objective of this study is to employ a molecular epidemiological approach to investigate the temporal association between viral infections and the development of KD.
Methods: We analyzed 2460 patients who underwent the FilmArray® Respiratory Panel test between April 2020 and September 2021.

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Results: Following the application of inclusion criteria, 2402 patients were categorized into KD (n = 148), respiratory tract infection (n = 1524), and control groups (n = 730). The KD group exhibited higher positive rates for respiratory syncytial virus (RSV), human rhinovirus/enterovirus (hRV/EV), parainfluenza virus (PIV) 3, and adenovirus (AdV) compared to the control group. Additionally, coinfections involving two or more viruses were significantly more prevalent in the KD group. Notably, RSV-positive, hRV/EV-positive, and PIV3-positive KD patients exhibited a one-month delay in peak occurrence compared to non-KD patients positive for corresponding viruses. In contrast, AdV-positive KD cases did not show a one-month delay in peak occurrence. Moreover, anti-RSV, anti-PIV3, and anti-AdV antibody-positive rates or antibody titers were higher in RSV-, PIV3-, and AdV-positive KD cases, respectively, compared to non-KD cases with the same viral infections.
Conclusion: Recent infection with RSV, PIV3, or AdV, occasionally in conjunction with other viruses, may contribute to the pathogenesis of KD as infrequent complications.

Introduction

Kawasaki disease (KD) is a childhood systemic vasculitis that primarily affects the coronary arteries.[1] Despite extensive research, the exact cause of KD remains unknown. However, its clinical manifestations and epidemiological characteristics, including age distribution, temporal and geographic clustering, seasonality, and preceding infectious outbreaks, strongly suggest infection as an etiological background.[2,3] Various infectious agents, including viruses, bacteria, and fungi, have been considered to be implicated in the onset of KD.[4,5]

In Europe and the USA, there has been an observed lag of 2—6 weeks between the peak of cases with Coronavirus disease 2019 (COVID-19) in communities and the emergence of cases with multisystem inflammatory syndrome in children (MIS-C), also known as Kawasaki-like disease.[6—8] Furthermore, children with MIS-C often exhibit laboratory-confirmed evidence (seropositivity in most cases and 10—30% polymerase chain reaction [PCR] positivity) of recent or acute infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As a result, MIS-C (Kawasaki-like disease) is now recognized as a post-infectious immune disorder associated with SARS-CoV-2.[6—8]

Recent epidemiological studies utilizing large-scale datasets have demonstrated a significant time lag (4—12 weeks) between certain viral infections and the onset of KD.[9—12] However, unlike the association between SARS-CoV-2 and MIS-C, patients with KD have yet to be definitively corroborated through laboratory testing to establish a link with acute or recent viral infections. In the present study, we aim to investigate the potential association between respiratory viruses and the development of KD employing a molecular epidemiological approach. This investigation involves the rapid detection of antigen by immunoassay and genome by PCR, and the assessment of specific antibody titers in individuals diagnosed with KD linked to viral infections.

Materials and methods

Participants

The study subjects included 2460 patients who underwent the FilmArray® Respiratory Panel test (FA-RP; BioMerieux Japan, Tokyo) at Fukuoka Children's Hospital from April 2020 to September 2021. Demographic, clinical, and laboratory data of the patients, as well as their FA-RP results, were extracted from their electronic medical records. Diagnosis, clinical categories, laboratory tests and sampling procedures are described in Supplementary Methods.

Statistical analyses

Statistical analyses were performed using JMP® Pro version 14.0 (SAS Institute, Cary, NC, USA). The positive rates for each respiratory pathogen in each group were compared by Poisson regression analysis to calculate incidence rate ratios (IRR) and their 95% confidence intervals (CI). Other comparisons were made using Fisher's exact test, Wilcoxon's rank sum test or Steel—Dwass test. A *P* value of less than 0.05 was considered statistically significant. Bonferroni's correction was used when necessary.

Ethics statement

This study was approved by the Ethics Committee of Fukuoka Children's Hospital (approval number 2021-1719). Informed consent for parents was substituted with an opt-out process, given the retrospective nature of this study in

the ethical committees' approval. Others are described in Supplementary Methods.

Results

Characteristics of KD, respiratory tract infection (RTI) and control groups

This study included a total of 2460 cases that underwent FA-RP testing and classified them into two groups based on the primary diseases: possible KD cases (n = 175) and non-KD cases (n = 2285). The non-KD cases were further divided into the RTI group and control group, based on the presence or absence of respiratory symptoms and/or signs, respectively. The final analysis included a total of 2402 cases, comprising the KD group (n = 148), the RTI group (n = 1524), and the control group (n = 730) (Supplementary Figure 1).

Supplementary Table 1 presents the demographic features of the KD, RTI, and control groups at the time of diagnosis. The median age was 25 months (range: 1–120 months) in the KD group, 20 months (range: 0–660 months) in the RTI group, and 39 months (range: 0–480 months) in the control group, respectively. Males accounted for 87 (58.8%) in the KD group, 824 (54.1%) in the RTI group, and 410 (56.2%) in the control group. Clinical and laboratory features of the KD Group are also shown in Supplementary Table 1.

Comparison of PCR detection rates for respiratory pathogens between the KD and control groups

Table 1A presents the comparison of the positive rates for each respiratory pathogen between the KD and control groups using Poisson regression analysis. The KD group exhibited significantly higher positive rates in FA-RP for respiratory syncytial virus (RSV) (IRR 2.11 [95% CI: 1.33–3.32], $P = 0.002$), human rhinovirus/enterovirus (hRV/EV) (IRR 1.28 [1.09–1.49], $P = 0.004$), and parainfluenza virus (PIV)-3 (IRR 1.70 [1.03–2.67], $P = 0.037$) compared to the control group. As for adenovirus (AdV), the KD group exhibited a tendency towards a higher positive rate (IRR 1.38 [0.93–1.95], $P = 0.10$) when compared to the control group.

The FA-RP data also revealed that the KD group had significantly lower rates in positive results for coronavirus OC43, PIV3 and RSV, than those in the RTI group (Supplementary Table 2). Thus, these data affirmed the reliability of the FA-RP-based analysis of viral infections in this study.

As shown in Table 1B, the frequencies of coinfections involving two or more viruses were significantly higher in the KD group (8.1%) compared to the control group (2.9%).

Table 1 Comparison of PCR detection rates for respiratory pathogens between the KD and control groups.

A. Data for each pathogen (virus)

Virus	KD group n = 148	Control group n = 730	IRR	95%CI	*P* value[a]
	n (%)	n (%)			
Adenovirus	10 (6.8%)	26 (3.6%)	1.38	0.93-1.95	0.10
Coronavirus NL63	2 (1.4%)	10 (1.4%)	0.99	0.39-1.93	0.99
Coronavirus OC43	2 (1.4%)	10 (1.4%)	0.99	0.39-1.93	0.99
hRV/EV	**51 (34.5%)**	**154 (21.1%)**	**1.28**	**1.09-1.49**	**0.004**
Parainfluenza virus 3	**7 (4.7%)**	**12 (1.6%)**	**1.70**	**1.03-2.67**	**0.037**
Parainfluenza virus 4	1 (0.7%)	4 (0.5%)	1.11	0.25-2.89	0.85
Respiratory syncytial virus	**9 (6.1%)**	**10 (1.4%)**	**2.11**	**1.33-3.32**	**0.002**

B. Data for the respective numbers of simultaneously detected pathogens

Number of simultaneously detected Viruses	**KD group** n = 148	**Control group** n = 730	***P* value**
	n (%)	n (%)	
0	83 (56.1%)	526 (72.0%)	<0.001[b]
1	53 (35.8%)	183 (25.1%)	
2	7 (4.7%)	19 (2.6%)	
3	5 (3.4%)	2 (0.3%)	

[a] Poisson regression analysis.
[b] Fisher's exact test.
CI, confidence interval; hRV/EV: human rhinovirus/enterovirus; IRR, incidence rate ratio; KD, Kawasaki disease; PCR, polymerase chain reaction.

In subsequent investigations, our attention was directed towards a closer examination of RSV, hRV/EV, PIV3, AdV, and their coinfections.

Temporal comparison of the occurrence of RSV-positive KD and Non-KD patients at Fukuoka Children's hospital and RSV surveillance data in Fukuoka Prefecture

To assess the temporal relationship between RSV infection and KD, we compared the occurrence of RSV-positive KD and non-KD cases to the prefectural RSV surveillance data in Fukuoka, Japan, where the study institution is located. RSV-positive non-KD cases in our institution and RSV surveillance data in Fukuoka Prefecture showed that the peak of RSV infection occurred in April 2021 (Fig. 1A, inset). In contrast, the peak of RSV-positive KD cases identified by FARP (n = 9) at Fukuoka Children's Hospital occurred in May 2021 (Fig. 1A, arrow), indicating a one-month delay in the onset of RSV-positive KD cases. Among the RSV-positive KD cases, two had laboratory (RSV antigen)-confirmed RSV infection one month prior to the onset of KD.

Anti-RSV antibody titers were compared among the following groups: RSV-positive KD cases, virus-negative KD cases, RSV-positive non-KD cases, and virus-negative non-KD cases. As depicted in the upper panel of Fig. 2A, the proportion of anti-RSV antibody-positive cases (neutralization test [NT] titer $\geq$4) was higher in RSV-positive KD cases (7/9, 77.8%) than in RSV-positive non-KD cases (4/9, 44.4%) and RSV-negative KD and non-KD cases (2/9, 22.2% each). However, the differences of antibody titers among the four groups did not reach statistical significance (Fig. 2A, upper). Consistently, anti-RSV antibody titers determined by enzyme-linked immunosorbent assay (ELISA) were found to be elevated in RSV-positive KD cases when compared to

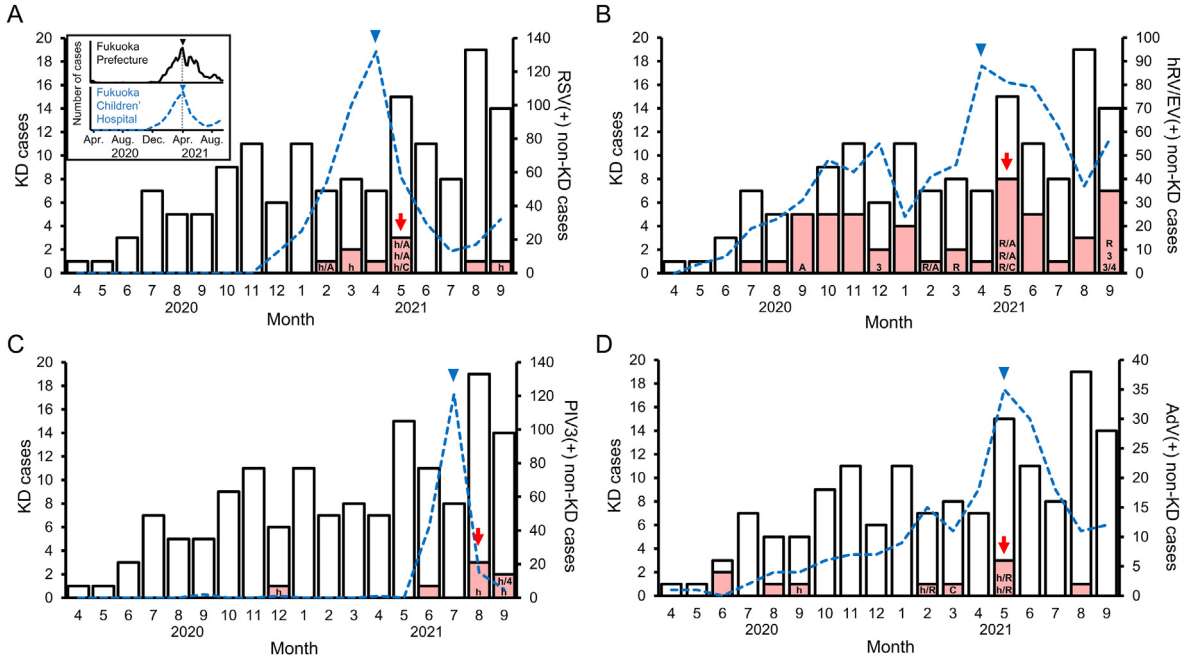

Figure 1. Temporal comparison of the occurrence of a certain respiratory virus-positive Kawasaki disease (KD) and non-KD cases. White bars represent the total monthly count of KD cases admitted to Fukuoka Children's Hospital. Pink areas represent the cases in which the respective viruses of interest were detected by PCR among all KD cases indicated by the white bars. A blue dotted line represents the number of respective virus-positive non-KD cases, with a blue arrowhead indicating their peak admission numbers. The letters shown within the pink area indicate the pathogens co-infected with the respective viruses of interest, as follows: A for adenovirus, C for Coronavirus NL63, h for hRV/EV, R for RSV, 3 for PIV3, and 4 for PIV4. A. The temporal trend of respiratory syncytial virus (RSV)-positive KD and non-KD cases. The inset shows a synchrony between the peak (blue arrowhead) in the monthly count of RSV-positive non-KD cases at Fukuoka Children's Hospital, and the peak (black arrowhead) in the weekly RSV patient count monitored by sentinel surveillance in Fukuoka Prefecture. RSV-positive non-KD cases at our institution (a blue dotted line) and RSV surveillance data in Fukuoka Prefecture both indicated an April 2021 peak in RSV activity. In contrast, the peak (red arrow) of RSV-positive KD cases at Fukuoka Children's Hospital occurred in May 2021. B. The temporal trend of human rhinovirus/enterovirus (hRV/EV)-positive KD and non-KD Cases. Pink areas denote KD cases with hRV/EV detected by PCR. The blue dotted line represents the number of hRV/EV-positive non-KD patients, with a blue arrowhead indicating their peak admission number. A red arrow indicates the peak in the number of hRV/EV-positive KD cases. C. The temporal trend of parainfluenza virus 3 (PIV3)-positive KD and non-KD Cases. Pink areas represent KD cases with PIV3 detected by PCR. The blue dotted line depicts the number of PIV3-positive non-KD patients, with a blue arrowhead indicating their peak admission numbers. A red arrow shows the peak in the number of PIV3-positive KD cases. Notably, there is a one-month delay in the peak of KD cases with PIV3. D. The temporal trend of adenovirus (AdV)-positive KD and non-KD Cases. Pink areas indicate KD cases with AdV. Interestingly, peaks for both AdV-positive non-KD (blue arrowhead) and KD cases (red arrow) were observed in May 2021.

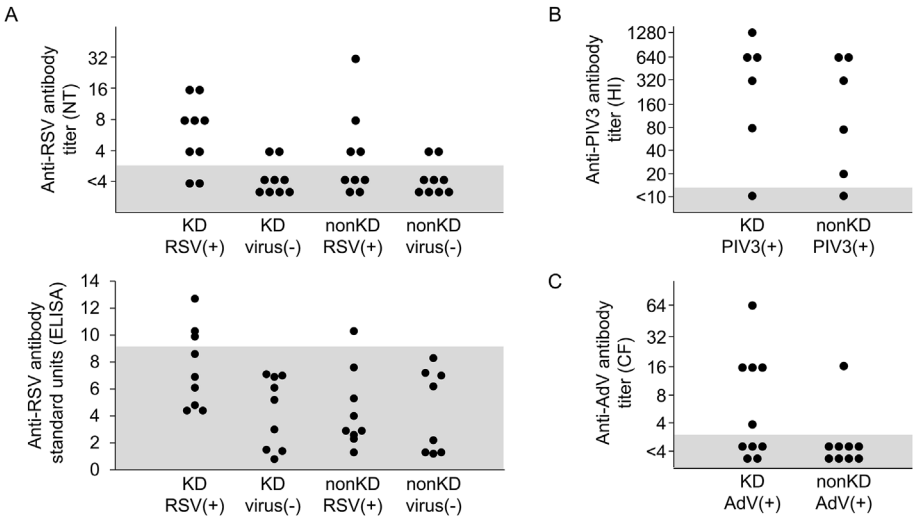

Figure 2. Anti-respiratory syncytial virus (RSV), anti-parainfluenza virus type 3 (PIV3) or anti-adenovirus (AdV) antibody titers in virus (RSV, PIV3 or AdV)-positive Kawasaki disease (KD) cases. A. Anti-RSV antibody titers in RSV-positive KD cases, virus-negative KD cases, RSV-positive non-KD cases, and virus-negative non-KD cases. **Upper Panel:** Neutralization Test (NT). The prevalence of positive anti-RSV antibodies (NT $\geq$ 4) was notably higher in RSV-positive KD cases (77.8%) compared to RSV-positive non-KD cases (44.4%) and RSV-negative KD and non-KD cases (2/9, 22.2% each). The differences of antibody titers among the four groups did not reach statistical significance (median [range]): RSV-positive KD cases, 8 [<4–16]; virus-negative KD cases, <4 [<4–4]; RSV-positive non-KD cases, <4 [<4–32] and virus-negative non-KD cases, <4 [<4–4]; $P = 0.13$, Fisher's exact test). **Lower Panel:** Enzyme-Linked Immunosorbent Assay (ELISA). Anti-RSV antibody titers determined by ELISA were found to be elevated in RSV-positive KD cases when compared to RSV-positive non-KD cases and virus-negative cases of both KD and non-KD (median [range]: 6.9 [4.4–12.7], 5.2 [0.8–7.1], 2.9 [1.3–10.3] and 6.2 [1.2–8.3], respectively, $P = 0.16$, Kruskal–Wallis test). A negative result fell below 9. The difference in antibody titers (ELISA) between RSV-positive KD cases and RSV-positive non-KD cases did not reach statistical significance. Two out of the nine RSV-positive KD cases exhibited negative anti-RSV antibody titers, as measured by both NT and ELISA (Fig. 2A). In these two RSV-positive KD cases, FA-RP testing revealed concurrent detection of hRV/EV in one, and hRV/EV plus AdV in the other. Notably, the one with RSV, hRV/EV and AdV displayed a positive anti-AdV antibody titer (CF x4). B. Anti-PIV3 antibody titers in PIV3-positive KD and non-KD cases. Anti-PIV3 antibody titers of PIV3-positive KD cases were higher than those of PIV3-positive non-KD cases, although the difference was not statistically significant (median [range]: 640 [<10–1280] and 80 [<10–640], respectively, $P = 1.00$, Fisher's exact test. C. Anti-AdV antibody titers in AdV-positive KD and non-KD cases. Anti-AdV antibody titers of AdV-positive KD cases were higher than those of AdV-positive non-KD cases, although the difference was not statistically significant (median [range]: 4 [<4–64] and <4 [<4–16], respectively, $P = 0.28$, Fisher's exact test).

RSV-positive non-KD cases and virus-negative cases of both KD and non-KD (Fig. 2A, lower).

Temporal comparison of the occurrence of hRV/EV-positive, PIV3-positive, or AdV-positive KD and non-KD patients at the study institution

There is a lack of surveillance data regarding the prevalence of patients with hRV/EV or PIV3 infection in Fukuoka Prefecture. As shown in the inset of Fig. 1A, we observed a synchrony between the peak in the monthly count of RSV-positive non-KD cases, identified through FA-RP testing at Fukuoka Children's Hospital (inset: dashed line, Fig. 1A), and the peak in the weekly RSV patient count monitored by sentinel surveillance in Fukuoka Prefecture (inset: solid line, Fig. 1A). Therefore, we have utilized the monthly count of hRV/EV-positive or PIV3-positive non-KD patients identified through FA-RP testing at Fukuoka Children's Hospital as a reliable proxy indicator for the prevalence of hRV/EV or PIV3 in the Fukuoka region (Fig. 1B and C). The surveillance data on the prevalence of AdV in Fukuoka Prefecture displayed only minor peaks (0.41, 0.33, and 0.67 cases per sentinel) in March, April, and May 2021, respectively. In such instances, AdV-positive non-KD cases at Fukuoka Children's Hospital seemed to be a more suitable indicator for the small AdV clusters in the vicinity of Fukuoka Children's Hospital (Fig. 1D).

We thus conducted a comparative analysis of the time series of hRV/EV-positive patients with KD and those without KD at our institution. The pink bar plots in Fig. 1B display the monthly count of hRV/EV-positive KD cases ($n = 51$) identified through FA-RP testing. In agreement with data for RSV-positive non-KD cases, the peak of hRV/EV-positive non-KD cases occurred in April 2021 (dashed line), whereas the peak for hRV/EV-positive KD patients was observed in May 2021 (arrow on the pink bar, Fig. 1B). However, it is crucial to recognize that the observed hRV/EV positivity in the KD group (34.5%) might not consistently exhibit KD-related positivity, as the control group also demonstrated a notable hRV/EV-positive rate of 21.1% (Table 1A). This suggests that hRV/EV infections in KD may not always be directly linked to KD development. Among the eight hRV/EV-positive KD cases in May 2021, three had coinfections with RSV, and ADV in two cases and RSV and coronavirus NL63 in one case.

We then conducted a similar analysis for PIV3-positive patients with KD and those without KD. Pink bars in Fig. 1C depict the monthly count of PIV3-positive KD cases (n = 7). Interestingly, the peak of PIV3-positive non-KD cases was recorded in July 2021, while that of PIV3-positive KD patients was noted in August 2021. Notably, among the seven cases with PIV3-positive KD, three exhibited simultaneous detection of hRV/EV, and one displayed co-detection of hRV/EV and PIV-4. For six of the seven PIV3-positive KD cases, we had access to sera for antibody studies. Five (83.3%) of the six cases tested positive for anti-PIV3 antibodies in both PIV3-positive KD and non-KD cases, whereas the anti-PIV3 antibody titers of PIV3-positive KD cases were higher than those of PIV3-positive non-KD cases, although the difference was not statistically significant (Fig. 2B). It is worth highlighting the noteworthy observation that PIV3-positive KD cases exhibited a one-month lag in peak occurrence, mirroring the temporal pattern observed in RSV cases.

Finally, we conducted the temporal analysis for AdV-positive patients with KD and those without KD. Fig. 1D illustrates the monthly count of AdV-positive KD cases (n = 10). Interestingly, the peaks of both AdV-positive non-KD and KD cases were observed in May 2021. While there was no one-month lag in peak occurrence between AdV-positive KD and non-KD cases, the potential for a lag of 1–2 weeks cannot be ruled out. Among these ten cases, four exhibited simultaneous detection of hRV/EV, three showed co-detection of RSV and one displayed co-detection of coronavirus NL63. Five (50.0%) of the ten cases tested positive for anti-AdV antibodies (CF), and the anti-AdV antibody titers of AdV-positive KD cases were higher than those of AdV-positive non-KD cases, although the difference was not statistically significant (Fig. 2C).

Differences in the clinical and laboratory characteristics of RSV-positive, hRV/EV-positive, PIV3-Positive, AdV-Positive or virus (RSV, hRV/EV, PIV3 or AdV)-positive KD in comparison to virus-negative KD

We analyzed the demographic, clinical, and laboratory features of RSV-positive (n = 9), hRV/EV-positive (n = 51), PIV3-positive (n = 7), AdV-positive (n = 10) or virus-positive (RSV, hRV/EV, PIV3 or AdV-positive, n = 63) KD cases in comparison to those of KD cases negative for all tested pathogens (n = 83). We used Fisher's exact test or Wilcoxon's rank sum test for these comparisons, as detailed in Table 2 and Supplementary Tables 3, 4, 5 and 6.

There were no significant differences in demographic, clinical or laboratory features between RSV- or AdV-positive KD cases and KD cases negative for all tested pathogens (Supplementary Tables 3 and 6). In contrast, hRV/EV-positive KD cases exhibited a significantly lower median C-reactive protein (CRP) value (Supplementary Table 4), and PIV3-positive KD cases displayed a significant delay in the initiation of IVIg treatment, when compared to KD cases negative for all tested pathogens (Supplementary Table 5). When the features of any virus-positive (RSV, hRV/EV, PIV3 or AdV-positive) KD cases (n = 63) were compared with those of KD cases negative for all tested pathogens, virus-

Table 2 Differences in the Clinical and Laboratory Features between Virus (RSV, hRV/EV, PIV3, or AdV)-Positive KD and Virus-Negative KD.

Characteristics	Virus positive cases n = 63	Virus-negative cases n = 83	*P* value
Age (month) at onset, median [range]	20 [1–85]	28 [3–120]	0.26
Sex (Male), n (%)	36 (57.1%)	51 (61.5%)	0.61
Day of illness at diagnosis, median [range]	5 [2–28]	4 [1–15]	0.89
Duration of fever (days), median [range]	6 [1–26]	6 [2–16]	0.52
Kobayashi score, median [range]	3 [0–10]	3 [0–10]	0.19
WBC count (10^9/L), median [range]	14.39 [5.65–30.65]	14.77 [5.19–30.85]	0.70
% Neutrophils, median [range]	67.5 [33.8–95.9]	74.8 [33.1–93.0]	0.23
Platelet count (10^9/L), median [range]	350 [146–746]	335 [85–697]	0.18
AST (IU/L), median [range]	38 [21–866]	32 [13–1281]	0.35
Sodium (mmol/L), median [range]	136 [130–140]	136 [127–140]	0.10
CRP (mg/L), median [range]	**68.1 [0.3–278.3]**	**77.8 [4.6–251.0]**	**0.040**
Treatment			
IVIg, n (%)	60 (95.2%)	79 (95.2%)	1.00
Day of 1st IVIg treatment, median [range]	5 [2–28]	5 [2–15]	0.96
Additional IVIg, n (%)	13 (20.6%)	22 (26.5%)	0.44
Steroid, n (%)	2 (3.2%)	2 (2.4%)	1.00
Infliximab, n (%)	3 (4.76%)	10 (12.1%)	0.15
Plasma exchange, n (%)	0 (0%)	1 (1.2%)	1.00
Incomplete KD, n (%)	6 (9.5%)	7 (8.4%)	1.00
Coronary artery lesions, n (%)	4 (6.4%)	4 (4.8%)	0.73

AdV, adenovirus; hRV/EV, human rhinovirus/enterovirus; IVIg, intravenous immunoglobulin; KD, Kawasaki disease; PIV3, parainfluenza virus 3; RSV, respiratory syncytial virus; WBC, white blood cell. Kobayashi score: score for IVIg resistance in Kawasaki disease; Sodium ≤133 mmol/L (2 points), Aspartate aminotransferase (AST) ≥100 int. units/L (2 points), C-reactive protein (CRP) ≥100 mg/L (1 point), Neutrophils ≥80 percent of the WBC count differential (2 points), Platelet count ≤300 × 10^9/L (1 point), Early diagnosis, with initial treatment at or before the fourth day of illness (2 points), Age ≤12 months (1 point).

positive KD cases showed significantly lower serum CRP levels than virus-negative ones (Table 2).

Discussion

Epidemiological and laboratory investigations have consistently confirmed a delayed correlation between SARS-CoV-2 infection and the emergence of MIS-C, often referred to as Kawasaki-like disease.[6—8] Similarly, KD has been associated with various viruses, including RSV, hRV, rotavirus, and norovirus in Korea,[9] as well as RSV, influenza A, influenza B, and hMPV in Chile,[10] with a 1—2 month lag. Recent epidemiological studies in Korea and the USA have also revealed significant links between KD and RSV, hRV, or varicella, with a delay of 1—3 months,[11] and between KD and RSV with a 2—5 week lag.[12] While these KD studies lacked laboratory-confirmed individual data for recent infections, our PCR and antibody data provide initial evidence supporting a notable connection between KD and RSV, PIV3 or AdV.

Due to infection-preventive measures, including physical distancing, hand washing, and mask-wearing, there was a near-absence of enveloped virus (RSV) epidemics over a one-year period (Supplementary Figure 2). However, in 2021, a substantial RSV epidemic occurred in Fukuoka, closely followed by a surge in RSV-positive KD cases, with a one-month lag. This study represents the first report demonstrating a temporal correlation (one-month lag) between RSV surveillance data in Fukuoka (PCR-confirmed RSV epidemic in controls) and PCR/antibody-confirmed RSV-positive KD cases. A temporal correlation was also observed between PCR-confirmed PIV3 epidemic in controls (non-KD cases) and PCR/antibody-confirmed PIV3-positive KD cases with one-month lag, and PCR-confirmed AdV epidemic in controls and PCR/antibody-confirmed AdV-positive KD cases without one-month lag, consistent to the reports that there was no one-month delay between infection and KD onset in AdV.[13] With regard to hRV/EV, analyzing the association between hRV/EV and KD development is challenging due to the limitations of multiplex PCR in differentiating between RV and EV. It is plausible that a specific RV or EV strain may be implicated in KD development. Alternatively, co-infection with hRV/EV could potentially accelerate KD development triggered by other pathogens, similar to the phenomenon observed in MIS-C.[14]

The seasonality of KD varies across countries, with higher incidence in winter in Japan, Canada, and Europe, and in summer in Taiwan and Korea.[2] This suggests that a variety of pathogens may be associated with KD development worldwide. The low recurrence rate of KD, ranging from 1.5% in Canada to 3—4% in Japan,[15] argues against a multiple etiologic theory for KD.[16] However, the infrequency of KD recurrence rates (1—4%) may be explained by the presence of antibodies to DAMPs, which could suppress KD development triggered by any pathogen. In the murine KD model induced by *Candida albicans* water soluble fraction, anti-DAMP (HMGB1) antibody has been shown to prevent coronary arteritis.[17] Notably, anti-DAMPs (oxidized LDLs) antibodies have been detected in healthy children, patients with febrile diseases, and patients with KD.[18—20] The presence of anti-DAMP (HSP7C) antibodies has also been reported in patients with KD.[21] Furthermore, IVIG preparations contain anti-DAMPs antibodies.[22]

A four-week delay between RSV or PIV3 infection and KD onset lends further support to the idea that KD is an immune-mediated disorder rather than a direct consequence of acute infection. Regarding MIS-C,[23,24] it has been suggested that antibody-dependent enhancement (ADE), autoantibodies, immune complexes, and co-infections or superinfections of viruses or bacteria may contribute to the delayed onset of this Kawasaki-like disease. ADE refers to a phenomenon in which non-neutralizing or sub-neutralizing virus-specific antibodies, or insufficient levels of neutralizing antibodies, enhance virus entry into cells through Fc receptors or canonical viral receptors.[25,26] Complement-mediated ADE may also be involved in the delayed onset of KD.[24] This process, in the absence or presence of complement, activates intracellular innate immune inflammasome pathways, leading to the production of pro-inflammatory cytokines and pyroptosis.

In severe COVID-19, it has been proposed that antibody-mediated SARS-CoV-2 infection of monocytes induces inflammatory cell death (pyroptosis) and inflammation.[27] Pyroptosis results in the release of large amounts of DAMPs, which subsequently trigger pyroptosis in endothelial and other cells.[28] This interplay might consequently amplify vascular inflammation, contributing to the development of Kawasaki-like disease and KD.[29] Autoantibodies cannot fully explain the characteristic features of KD, such as age distribution, clustering, seasonality, self-limitedness, and rare recurrence. Immune complex depositions have not been detected in KD vasculitis lesions.[30] Further, it is unlikely that autoantibodies and immune complexes play a major role, as there is no delay between infection and KD onset in *Yersinia pseudotuberculosis*.[31] Viruses and bacteria often activate distinct inflammasome pathways.[32,33] Co-infection or superinfection with viruses or bacteria may induce synergistic hyperactivation of the innate immune system,[12,33] and pyroptosis.[34—36] Pyroptosis might explain the association of KD with an array of infectious agents, irrespective of the duration of the incubation period or the presence or absence of ADE.

While infection-preventive behaviors significantly reduced respiratory and gastrointestinal infections through the inhibition of contact and/or droplet transmission, they did not reduce the number of KD cases more than half.[37—39] An analysis based on time series data from France suggests that approximately 35% of KD cases could potentially be attributed to seasonal pathogens such as AdV, norovirus, and RSV.[40] Our molecular epidemiological study in Fukuoka has demonstrated that RSV, PIV3 and AdV, sometimes with other viruses, may contribute to the pathogenesis of KD as infrequent complications, similar to a Kawasaki-like syndrome observed in fewer than 1 in 1000 children following exposure to SARS-CoV-2.[6]

In conclusion, our findings support the theory that KD is an immune-mediated disorder rather than a direct consequence of acute infection. Given the variation in antibody profiles against pathogens across different countries and regions due to factors such as exposure history, vaccination rates, and genetic predisposition, it is reasonable to expect that the pathogens that trigger KD may vary among nations and regions.[2] We suggest that seasonal viruses and bacteria

play a role in the development of 30—50% of KD. However, the precise mechanism by which these viruses contribute to KD is still unknown. Further investigation is imperative to identify what pathogens are implicated in KD and delineate the mechanisms through which they precipitate the disease.

Data statement

All the data in this study are available in the main manuscript and supplementary materials. Supplementary data are available at the URL: https://archive.iii.kyushu-u.ac.jp/public/aPpBw56I9B3_7mIxc4SJ_VOWHZZ4A2REXxAOrB3XqxIi.

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Declaration of generative AI in the writing process

During the preparation of this work, the author used [ChatGPT-3.5/Open AI, and Bard/Google] in order to check grammar and spelling, and to improve readability. After using these tools/services, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

CRediT authorship contribution statement

Kentaro Marutani: Formal analysis, Writing — original draft. **Kenji Murata:** Data curation, Formal analysis, Writing — original draft, Writing — review & editing. **Yumi Mizuno:** Formal analysis, Investigation. **Sagano Onoyama:** Investigation. **Takayuki Hoshina:** Investigation, Resources. **Kenichiro Yamamura:** Formal analysis. **Kenji Furuno:** Formal analysis. **Yasunari Sakai:** Supervision, Writing — review & editing. **Junji Kishimoto:** Data curation, Formal analysis. **Koichi Kusuhura:** Supervision, Writing — review & editing. **Toshiro Hara:** Conceptualization, Formal analysis, Resources, Supervision, Writing — original draft, Writing — review & editing.

Declaration of competing interest

The authors declare no financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

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