Hygienic behaviors during the COVID-19 pandemic may decrease immunoglobulin G levels: Implications for Kawasaki disease

Hiromi Yamaguchi1,2, Masaaki Hirata1, Kuniya Hatakeyama1, Ichiro Yamane1, Hisashi Endo4, Hiroe Okubo1, Yoshimi Nishimura1, Yoshiro Nagao1*

1 Department of Pediatrics and Neonatology, Fukuoka Tokushukai Hospital, Kasuga, Fukuoka, Japan, 2 Department of Pediatrics, Fukuoka University, Fukuoka, Japan

* ng999214@iis.u-tokyo.ac.jp

Abstract

Background

Due to the coronavirus disease 2019 (COVID-19) pandemic, hygienic behaviors became a new norm since January 2020. The hygiene hypothesis predicts that an excessively hygienic environment may adversely affect human health.

Objective

We quantified the effect of COVID-19 on immunologic parameters linked to the hygiene hypothesis.

Methods

We examined age-specific levels of total nonspecific immunoglobulin G (IgG) and IgE in individuals who visited Fukuoka Tokushukai Hospital between 2010 and 2021. Pre-COVID (2010–2019) and COVID (2020–2021) periods were compared.

Results

IgG levels steadily decreased throughout Pre-COVID period. IgG levels fell abruptly from the pre-COVID period to the COVID period in all age groups (P = 0.0271, < 0.3 years; P = 0.0096, 0.3–5 years; P = 0.0074, ≥ 5 years). The declines in IgG in < 0.3 years and that in ≥ 5 years accelerated during the COVID period. IgE levels were seasonal, but did not change noticeably from the pre-COVID to COVID period. IgG levels recorded for patients with Kawasaki disease (KD) (mean 709 mg/dL) were significantly lower than for matched control subjects (826 mg/dL) (P<0.0001).

Discussion

Hygienic behaviors during the COVID-19 outbreak decreased the chance of infection, which may explain the decreases in IgG levels in children and adults. Neonatal IgG declined, possibly because of the decrease in maternal IgG.
Conclusion
Hygienic behaviors decreased the IgG levels in all age groups, from neonates to adults. This downturn in IgG may lead to vulnerability to infections as well as to KD.

Introduction
The new variant coronavirus (SARS-CoV-2) was reportedly brought to Japan in January, 2020 [1]. Following the global and domestic spread of coronavirus disease 2019 (COVID-19), the Japanese public voluntarily took precautional measures, such as wearing face coverings, and distancing from each other. In March 2020, 67% of the Japanese wore face masks in public places, at a higher rate than in Western countries (e.g. 42% in Spain, 17% in the US and 6% in the UK) [2]. The Japanese government declared a state of emergency repeatedly and requested that the public refrain from non-urgent travel and gatherings. More rigorous regulations were enforced in populated prefectures including Fukuoka prefecture (population 5 million), where our hospital is located.

It is hypothesized that the strict hygiene regulations practiced during the COVID-19 pandemic may affect microbiota that inhabit humans and cause immunological problems [3]. This concern is based upon the long-held hypothesis that as the environment becomes more hygienic, decreasing exposure to infections can adversely affect human health [4]. One study found that hygienic conditions are correlated with an increased risk for inflammatory bowel disease [5]. It was suggested that targeted hygiene against pathogens and sharing essential microbes are both important [6].

Serum Immunoglobulin E (IgE) is an indicator of allergic propensity. Numerous studies revealed that insufficient exposure to microbial diversity during the early days of life induces high IgE levels, which subsequently leads to autoimmune and allergic disorders [7–14]. In contrast to IgE, whose role in protective immunity is not fully understood, Immunoglobulin G (IgG) is a critical mediator of infection immunity. However, there are few studies of IgG in the context of the hygiene hypothesis. In wild animals, nonspecific total IgG levels are higher than in captive animals [15, 16]. Nonspecific total IgG, which is frequently measured in laboratory tests in Japan, could potentially be a useful indicator of nonspecific infection immunity.

The hygiene hypothesis has been proposed as a possible explanation for Kawasaki disease (KD) [17–19]. KD is a febrile pediatric illness with mucocutaneous manifestations. KD most frequently affects children < 5 years of age in Japan and in many other regions/countries [20–22]. Importantly, KD is associated with high rates of potentially fatal coronary complications [23–25]. Although KD was first reported in 1967 [26], its etiology remains unknown. Numerous study results suggest that KD has an infectious etiology. For example, in Japan, KD incidence has a bimodal seasonality that is identical to pediatric viral infections [27]. KD is rare at < 3 months of age [28], which indicates the presence of maternal immunity. KD cases cluster in space and time [29–31]. B cell selection in patients with KD is consistent with an infectious origin rather than an autoimmune origin [32]. Meanwhile, a genetic preposition to KD has been found [33–36]. Therefore, it is widely accepted that KD is triggered when a genetically susceptible individual is infected by an as yet undetermined microbe(s) [37].

In this study, we examined whether the rigorous hygiene regulations imposed during the COVID-19 pandemic affected immunity in the human population. We also attempted to develop a hypothesis about the relationship between the change in population immunity and health outcomes, in particular for KD epidemiology.
Results

Human movement and distancing in the study area

Fig 1 shows that people in Fukuoka prefecture avoided public places and workplaces to the greatest degree through the first half of 2020. This avoidance culminated in May 2020 with a > 50% reduction in visits to transit stations. Throughout 2020 and 2021, the degree of this indicator fluctuated, but remained 20% below baseline. People started pre-emptive distancing well before states of emergency were declared.

Measurement of IgG and IgE

Between January 2010 and December 2021, total nonspecific IgG and IgE were tested 33,107 and 6,530 times, respectively. To avoid the effects of duplicate sampling on the analysis, we used only the first measurement for each individual (19,744 and 5,433 individuals for IgG and IgE, respectively. Table 1).
IgG and age

IgG level had a mostly positive correlation with age (Spearman’s R = 0.3962, \( P < 0.0001 \), \( n = 19,744 \), Fig 2A). IgG levels declined from birth to 3–4 months of age; this change reflected waning maternal immunity (Fig 2B). Subsequently, IgG levels steeply surged up to 5 years of age. Based on this result, in the analysis of IgG we classified individuals into three age groups: infants and neonates < 0.3 years; children between 0.3 and 5 years; individuals \( \geq 5 \) years.

Temporal shift in IgG

Annual IgG levels declined over the years in all age groups (Fig 3). There was a statistically significant decrease in IgG from 2019 to 2020 in all age groups (\( P = 0.0096 \) in < 0.3 y; \( P = 0.0271 \) between 0.3 and 5 y; \( P = 0.0074 \) in \( \geq 5 \) y, Mann-Whitney-Wilcoxon test) (Fig 3A). Fig 3(B) presents this result using a finer temporal resolution; IgG declined dramatically throughout 2020 in early infants (< 0.3 year) and in individuals \( \geq 5 \) years. These findings were supported by the results of regression analyses (Table 2): the downward temporal trends in IgG were statistically significant in all age three groups during the pre-COVID period (i.e., 2010–2019). In contrast, the decline in IgG was significant only in early infants and neonates (< 0.3 years) and individuals \( \geq 5 \) years during the COVID period (i.e., 2020–2021). This temporal trend was significant even after including age in the multivariate analysis. The results for the multivariate coefficients (Table 2) indicated that IgG decreased by 2.0 mg/dL (< 0.3 years), 9.2 mg/dL (0.3–5 years), and 14 mg/dL (\( \geq 5 \) years) per year, during the pre-COVID period. During the COVID period, IgG declined annually by 43 mg/dL (< 0.3 years) and 48 mg/dL (\( \geq 5 \) years). IgG did not decrease significantly in the 0.3–5 years age group during the COVID period. We repeated these analyses after transforming IgG data into a normal distribution using the Box-Cox method [39], and qualitatively validated the results (S1 Table). Subdivision of the age class \( \geq 5 \) years showed that, during the COVID period, the IgG declined by 42.1 mg/dL per year in 5–50 years age group (\( P = 0.0138 \), \( n = 1,325 \)) and 42.5 mg/dL per year in \( \geq 50 \) years (\( P = 0.1521 \), \( n = 952 \)) (S2 Table).

The results presented in Fig 3(B) suggested that IgG fluctuated over time. Periodicity analysis found that the cycle of this periodicity was much longer than 2.5 years (S1 Fig). Because the study period was only 12 years, we did not consider effects of this fluctuation in the statistical analyses.

### Table 1. Numbers of individuals for whom total nonspecific IgG or IgE were measured between 2010 and 2021.

| Year | < 0.3 y | 0.3–5 y | \( \geq 5 \) y | Total | < 5 y | 5–20 y | \( \geq 20 \) y | Total |
|------|---------|---------|-------------|-------|------|-------|-----------|-------|
| 2010 | 660     | 133     | 473         | 1266  | 90   | 111   | 105       | 306   |
| 2011 | 583     | 73      | 454         | 1110  | 126  | 82    | 236       | 444   |
| 2012 | 650     | 122     | 681         | 1453  | 269  | 109   | 244       | 622   |
| 2013 | 643     | 167     | 535         | 1345  | 318  | 74    | 128       | 520   |
| 2014 | 688     | 189     | 725         | 1602  | 268  | 103   | 129       | 500   |
| 2015 | 743     | 314     | 873         | 1930  | 243  | 105   | 133       | 481   |
| 2016 | 807     | 361     | 1063        | 2231  | 190  | 88    | 92        | 370   |
| 2017 | 727     | 249     | 865         | 1841  | 169  | 82    | 87        | 338   |
| 2018 | 602     | 326     | 699         | 1627  | 177  | 69    | 171       | 417   |
| 2019 | 613     | 322     | 784         | 1719  | 140  | 89    | 228       | 457   |
| 2020 | 413     | 229     | 980         | 1622  | 101  | 78    | 246       | 425   |
| 2021 | 353     | 348     | 1297        | 1998  | 144  | 91    | 318       | 553   |
| Total| 7482    | 2833    | 9428        | 19744 | 2235 | 1081  | 2117      | 5433  |

https://doi.org/10.1371/journal.pone.0275295.t001
The correlation of IgE values with age was significant (Spearman’s R = 0.5368, P < 0.0001, n = 2,988). IgE is not transferred transplacentally. IgE steeply increased as age increased, up to 20 years of age. Therefore, for the analyses of IgE, individuals were classified into three age classes: < 5 years, 5–20 years, and ≥ 20 years.

Fig 2. IgG and age. Nonspecific total IgG values, which were tested between January 2010 and December 2021 in Fukuoka Tokushukai Hospital, were plotted against ages of individuals, for all age groups (a), and for individuals < 10 years of age (b). The red lines represent the results for Lowess smoothing with a 14-day bandwidth.

https://doi.org/10.1371/journal.pone.0275295.g002

IgE and age

The correlation of IgE values with age was significant (Spearman’s R = 0.5368, P < 0.0001, n = 2,988). IgE is not transferred transplacentally. IgE steeply increased as age increased, up to 20 years of age. Therefore, for the analyses of IgE, individuals were classified into three age classes: < 5 years, 5–20 years, and ≥ 20 years.
Fig 3. Temporal changes in IgG. (a) IgG values averaged for each year are presented for three age groups of individuals of < 0.3 years (diamond), 0.3–5 years (triangle) and ≥ 5 years (square). Statistical comparisons between IgG values reported in one year and those reported in an adjacent year were performed using sum of rank tests (i.e., Mann-Whitney-Wilcoxon test). * P<0.05, ** P<0.01, *** P<0.001. (b) IgG values from these three age groups were smoothed using the Lowess method with a 14-day bandwidth.

https://doi.org/10.1371/journal.pone.0275295.g003
Temporal shift in IgE

When IgE levels were smoothed over the time in the analysis, there was seasonality in all age classes (Fig 4). To identify periodicities, we performed periodogram analysis of each age class (Fig 5). Periodicity with a one-year cyclicity (i.e., seasonality) was found in children <5 years of age. However, seasonality was more obscure in the older classes. We used regression analysis to identify temporal trends in IgE. A linear combination of sine and cosine transformations of time was included in multivariate analyses to represent seasonality. Only the results of the multivariate analysis performed for the <5 years age class and in the pre-COVID period indicated that there was a significantly negative temporal trend (Table 3). A multivariate analysis revealed that the sine term had a significant correlation with IgE, which indicated the presence of seasonality. We repeated these regression analyses by transforming IgE values into a normal distribution [39], and validated the results qualitatively (S3 Table). Taken together, we did not find unequivocally that COVID-19 affected IgE in the population (S2 Fig).

Comparison of IgG between patients with KD and matched control individuals

IgG levels were compared between 314 children with KD and an equivalent number of matched control children (Fig 6). In both groups, the prevalence of female subjects was 40.8%. The mean age at IgG testing was 2.63 years for the group of patients with KD and 2.67 years for the matched control group. The mean IgG was 709 mg/dL (95% confidence interval: 682–736 mg/dL) for the group of patients with KD and 826 mg/dL (789–862 mg/dL) for the matched control group (P<0.0001, Wilcoxon’s matched-pairs signed-ranks test).

To consider the possibility that this lower IgG in KD patients may have biased our previous analysis, we repeated the regression analysis (Table 2) by excluding the patients with KD. However, the result was not affected qualitatively (S4 Table).

Table 2. Linear regression coefficients to explain IgG in different age groups.

| Period                | < 0.3 years | 0.3–5 years | ≥ 5 years |
|-----------------------|-------------|-------------|-----------|
| 1. Pre-COVID (2010–2019) |             |             |           |
| 1.1 Univariate        |             |             |           |
| Time (years)          | - 4.81      | - 10.0      | - 16.8    |
| Coefficient           | P = 0.0002  | P < 0.0001  | P < 0.0001|
| Adjusted R²           | 0.1843      | 0.0077      | 0.0070    |
| Coefficient           | P = 0.0002  | P < 0.0001  | P < 0.0001|
| 1.2. Multivariate     |             |             |           |
| Time (years)          | - 2.01      | - 9.2       | - 13.6    |
| Coefficient           | P = 0.0895  | P < 0.0001  | P < 0.0001|
| Age† (years)          | - 2770      | 94          | 5.18      |
| Coefficient           | P < 0.0001  | P < 0.0001  | P < 0.0001|
| Adjusted R²           | 0.1784      | 0.1841      | 0.0656    |
| Coefficient           | P < 0.0001  | P < 0.0001  | P < 0.0001|
| 2. COVID (2020–2021)  |             |             |           |
| 2.1 Univariate        |             |             |           |
| Time (years)          | - 82.1      | 4.23        | - 48.8    |
| Coefficient           | P < 0.0001  | P = 0.8261  | P = 0.0039|
| Adjusted R²           | 0.0305      | -0.0017     | 0.0032    |
| Coefficient           | P < 0.0001  | P = 0.8261  | P = 0.0039|
| 2.2. Multivariate     |             |             |           |
| Time (years)          | - 42.6      | 13.1        | - 48.1    |
| Coefficient           | P = 0.0029  | P = 0.4395  | P = 0.0029|
| Age (years)           | - 2455      | 99.4        | 5.14      |
| Coefficient           | P < 0.0001  | P < 0.0001  | P < 0.0001|
| Adjusted R²           | 0.3096      | 0.2292      | 0.0849    |
| Coefficient           | P < 0.0001  | P < 0.0001  | P < 0.0001|

*Time that elapsed from the beginning of study period to the date of IgG measurement (in years).
† Age at the date of IgG measurement. For both time and age, the minimal temporal resolution used was day, while the unit of time is expressed in year.

https://doi.org/10.1371/journal.pone.0275295.t002
We examined whether changes in hygiene behavior during the COVID-19 outbreak affected nonspecific immunity represented by total IgE and IgG. IgE levels presented seasonality, being consistent with previous reports [40, 41]. However, there was no unambiguous result which supported presence of temporal trend in IgE or effect of COVID-19 on IgE levels. In contrast,

Fig 4. Temporal changes in IgE. IgE values were recorded for each age group, (a) < 5 years, (b) 5–20 years, and (c) ≥ 20 years, and were smoothed using the Lowess method (bandwidth = 14 days).

https://doi.org/10.1371/journal.pone.0275295.g004

Discussion

We examined whether changes in hygiene behavior during the COVID-19 outbreak affected nonspecific immunity represented by total IgE and IgG. IgE levels presented seasonality, being consistent with previous reports [40, 41]. However, there was no unambiguous result which supported presence of temporal trend in IgE or effect of COVID-19 on IgE levels. In contrast,
IgG decreased slowly but steadily since at least 2010. The rate of decrease in IgG abruptly accelerated in 2020, coinciding with the COVID-19 pandemic. This drop in IgG was most prominent in populations > 5 years of age and in neonates and early infants < 0.3 years of age. These results suggested that hygienic behaviors (e.g., face coverings and distancing) reduced opportunities for infection with microbes, leading to the decline in IgG. Because the mean half-life of IgG is 21 days [42], lack of infection would most likely decrease the IgG without delay. The mother’s IgG is transferred to the fetus during the last few months of pregnancy [43]. The IgG levels in mothers and in umbilical cord blood are highly correlated [44]. Therefore, a downturn in maternal IgG would be reflected in a change in neonatal IgG. In contrast, children between 0.3 years and 5 years would likely not have been affected by the reduction in infection because they were not required to wear face coverings during the COVID-19 outbreak in Japan. Interestingly, there was a significant IgG decline in all age groups from 2016 to 2017 (Fig 3A). This decrease may have been related to an increase of the face mask production in Japan which occurred in 2015 [45].

The KD incidence in Japan decreased by 35% in 2020 from the pre-COVID years [46], implying that the hygiene behaviors may have affected this illness. Therefore, it would be worthwhile to discuss the potential impact of the decreasing IgG on KD. The number of KD cases has been increasing rapidly in Japan and in many developed countries/regions [47, 48].

Table 3. Regression analysis to explain IgE in different age groups.

| 1. Pre-COVID (2010–2019) | < 5 years | 5–20 years | ≥ 20 years |
|-------------------------|-----------|------------|-----------|
| n = 1990 | n = 912 | n = 1553 |
| 1.1 Univariate | | | |
| Coefficient | P | Coefficient | P | Coefficient | P |
| Time (year) | -7.47 | 0.1285 | -9.97 | 0.5106 | 3.93 | 0.9009 |
| Adjusted R² | 0.0007 | 0.1285 | -0.0006 | 0.5106 | -0.0006 | 0.9009 |
| 1.2. Multivariate | | | |
| Coefficient | P | Coefficient | P | Coefficient | P |
| Time (year) | -10.1 | 0.0335 | -12.0 | 0.4287 | 5.46 | 0.8628 |
| Age (year) | 113 | 0.0001 | 33.0 | 0.0052 | 5.14 | 0.3134 |
| sin(2π×Time) | -57.8 | 0.0005 | -158 | 0.0087 | -297 | 0.0257 |
| cos(2π×Time) | -19.0 | 0.2693 | 34.8 | 0.5828 | -250 | 0.0726 |
| Adjusted R² | 0.0756 | 0.0001 | 0.0125 | 0.0039 | 0.0030 | 0.0721 |
| 2. COVID (2020–2021) | | | |
| n = 245 | n = 169 | n = 564 |
| 2.1 Univariate | | | |
| Coefficient | P | Coefficient | P | Coefficient | P |
| Time | 106 | 0.1704 | -69.3 | 0.7262 | -304 | 0.2625 |
| Adjusted R² | 0.0036 | 0.1704 | -0.0052 | 0.7262 | 0.0005 | 0.2625 |
| 2.2. Multivariate | | | |
| Coefficient | P | Coefficient | P | Coefficient | P |
| Time (year) | 33.3 | 0.6663 | -187 | 0.3660 | -235 | 0.4063 |
| Age (year) | 143 | 0.0001 | 108 | 0.0002 | 9.93 | 0.2000 |
| sin(2π×Time) | -133 | 0.0246 | -146 | 0.3710 | 174 | 0.4367 |
| cos(2π×Time) | -107 | 0.0697 | 320 | 0.0410 | -48.9 | 0.8167 |
| Adjusted R² | 0.0965 | 0.0001 | 0.0779 | 0.0017 | -0.0009 | 0.4771 |

*Time elapsed from the beginning of study period to the date of IgE measurement (by year).
† Age at date of IgE measurement. For both time and age, the minimal temporal resolution was day, while the unit of time is expressed in year.

https://doi.org/10.1371/journal.pone.0275295.t003
This constant increase in developed countries prompted discussion about the hygiene hypothesis [17]. Consistent with the hypothesis, KD risk is positively correlated with higher income, urbanization, and smaller family size [18, 49]. Lee hypothesized that the etiology of KD is dysregulated early B cell development under reduced microbial exposure [19]. Study findings indicate that before intravenous immunoglobulin (IVIG) administration, serum IgG levels in patients with KD are lower than in individuals without KD [50]. To our knowledge, our study was the first to quantify this phenomenon using accurately matched control subjects. Whether this low IgG level in patients with KD is a consequence of the systemic inflammation associated with KD or a predisposing factor for development of the illness, or both, remains unknown. Although IVIG is the mainstay of KD treatment, the mechanism for how IVIG cures KD is unknown. It was hypothesized that the IgG in IVIG suppresses excessive immune reactions or neutralizes causative microbes, or both [51]. A low serum IgG level before the first IVIG treatment predicts unresponsiveness to the treatment [50, 52], possibly because IVIG fails to sufficiently elevate the IgG level [53]. A lower level of IgG after the initial IVIG administration predicts a risk of coronary aneurysm [54–56]. Therefore, it is likely that a child with KD who has a low IgG level before IVIG treatment is at higher risk for the development of coronary aneurysms.
lower IgG may be more prone to develop KD (or at least complicated KD) than children with higher IgG, when triggered by one or more unknown aetiologic agents. This hypothesis may explain why preterm birth is associated an elevated risk for KD [57], because IgG levels in preterm babies are lower than in term babies (S3 Fig) [43]. In a sense, the “window” of pediatric ages at which children are vulnerable to KD (or at least IVIG-refractory KD) may correspond to a hypothetical threshold of IgG (Fig 7). This study revealed that IgG levels in all age groups constantly decreased over at least a decade, and this downward trend accelerated during the COVID-19 outbreak. This finding suggested that the “window” of age vulnerable to KD expands into both younger and older ages. This mechanism may also explain why the age distribution of KD in Japan has expanded in both directions since 1970 [49]. In 2020, the number of patients with KD decreased in Japan [46, 58]. However, our window hypothesis predicts that the incidence of KD may increase after a transient decrease, in a way that has been observed for other infectious diseases [59].

The results of one study suggested that a decline in the total fertility rate in Japan caused an increase in KD incidence, with a lag of 15 years [49] (S4 Fig). Total fertility rate has also been
found to be a surrogate for force of infection of pediatric infectious diseases [27]. Based on these findings, we hypothesized that a decline in the force of infection increased KD incidence after a long delay. The rate of decrease in IgG predicted in this study may seem too small to cause any change in KD epidemiology. However, this very slow rate may explain why it takes 15 years for a decline in fertility rate (and hence, in the force of infection) to increase KD incidence [49].

The new hygienic norm has become prevalent in developed and developing countries. This behavioral change may decrease IgG levels in diverse regions, and result in unexpected consequences for human health. For example, KD may be expanded into previously unaffected regions and ages. Because of decreasing IgG in children, the current standard dose of IVIG (2 g/kg) may become insufficient for an increasing number of patients with KD.

This study had some limitations. The data were derived retrospectively from a single hospital and may not necessarily represent a general, healthy population. The time elapsed during the COVID-19 period was only 2 years. This short period limited the statistical power to detect any temporal trends from this period or to identify a change in the clinical picture of KD, compared with the pre-COVID era. In our dataset, 50.1% of infants < 0.3 years of age were neonates admitted to the neonatal intensive care unit (NICU). However, the proportion of extremely preterm and/or low birthweight babies admitted to the NICU steadily decreased in recent years (S5 Fig). Because these babies have had a limited duration to receive maternal IgG transplacentally [43], their IgG levels are low (S3 Fig). Therefore, the analysis most likely underestimated the rate in IgG decrease in the population < 0.3 years of age. Despite these caveats, we identified a significant downturn in the IgG in this youngest population. This result supported the robustness of our result.

Although indiscriminate extinction of commensal organisms and common harmless microbes would likely induce unfavorable effects on regulatory immunity [60], targeted hygiene should still be maintained [61]. The magnitude of distancing varies greatly between countries/regions. These heterogeneities provide an opportunity to correlate socio-behavioral changes to human immunological parameters (e.g. total IgG) and health outcomes. The results may provide information that can be used to develop effective targeted hygiene measures. Future multi-region long-term studies are warranted to predict the effects of the global COVID pandemic on health problems, including KD.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee for Fukuoka Tokushukai Hospital, with reference 220302. The Ethics Committee waived the requirement for informed consent because of the retrospective nature of this study. All data were fully anonymized before the analysis.

Community mobility

Indicators for time spent in categorized locations, which started 15 February 2020, were downloaded from "COVID-19 Community Mobility Reports–Google" [38]. The baseline represented a median value for day of the week, recorded between 3 January and 6 February 2020. We examined three categories for location (i.e., transit stations, workplace, and residential area), smoothed over time using the Lowess method.

Study site and period

We extracted clinical records and laboratory data records from the electronic medical recording system of Fukuoka Tokushukai Hospital. This system began operation in August 2009. We
used the dates from January 2010 to December 2021. Medical records for 896,381 individuals were stored in the system on December 2021.

**IgG measurement**

IgG was measured using a Clinical Analyzer 7180® (Hitachi High-Tech, Japan) until August 2014. A TBA-c16000® analyzer (Canon Medical Systems, Japan) was used thereafter. Measurement accuracy was validated once per year by the Medical Society of Fukuoka Prefecture (MSFP). At each validation, the laboratory of our hospital measured two to three samples delivered from the MSFP and returned the values to the MSFP. During the study period, our hospital passed all the validations with "A-level" scores. This result indicated that the results were within 2 standard deviations of values reported from all hospital laboratories in Fukuoka prefecture.

**IgE measurement**

Total nonspecific IgE was measured by BML Inc. (Tokyo, Japan) until March 2015, and by the SRL Corporation (Tokyo, Japan) thereafter.

**Regression analysis to detect temporal trends**

We used univariate or multivariate linear regression analysis to identify temporal trends in IgG and IgE. Day was the level of resolution for the age and time variables, while unit of time is expressed in year.

**Statistical test to compare IgG between adjacent years**

We compared IgG and IgE values between adjacent years (e.g., 2009 vs 2010) using Mann-Whitney-Wilcoxon tests.

**Periodicity analysis**

The values for IgG or IgE were averaged for each quarter of each year, in each age class. The resulting time-series data were analyzed using the periodogram method to identify periodicity [62].

**Comparison of IgG between patients with KD and matched control children**

We screened 476 patients with KD who had their first clinical diagnosis for KD, between 2010 and 2019 (i.e., pre-COVID era), and for whom IgG was tested before initiation of IVIG treatment. Here, we selected patients with KD from only the pre-COVID period to exclude possible SARS-CoV-2-induced illness, which mimics KD [63–65]. IgG levels were compared between children with KD and the control children. A control child without KD was matched to a case with KD, in terms of sex, age, and date of IgG measurement. The difference in age and that in date of IgG measurement between a patient with KD and a matched control subject were both within 6 months. Using in-house software [66], we matched 314 patients with KD to an equivalent number of control children.

**Statistical software**

We used Stata/SE 13.1 (TX, USA) for the statistical analysis. Statistical significance was defined as P<0.05.
Datasets
The datasets used in the present study are available from https://www.kaggle.com/datasets/yoshironagao/hygienic-behaviors-during-the-covid-stata-files.

Supporting information

S1 Fig. IgG periodicity. IgG values were averaged for each quarter of each year between 2010 and 2021, for each age group. Periodograms were created for these time-series datasets. Seasonality (i.e., one-year cycles) was not found for each age group.
(EPS)

S2 Fig. Temporal change in IgE. IgE values were averaged for each year, in individual age groups. IgE values recorded in one year were compared to those in the adjacent year using rank sum tests (i.e., Mann-Whitney-Wilcoxon tests).
(EPS)

S3 Fig. IgG and gestational age in neonatal intensive care unit (NICU). Between 2010 and 2021, 3,739 neonates were admitted to the NICU. Among these neonates, this number includes 3,638 neonates for whom IgG was measured within 7 days from birth. IgG was highly correlated with gestational age (adjusted \(R^2 = 0.5447, P < 0.0001\)).
(EPS)

S4 Fig. Temporal negative correlation between total fertility rate and KD incidence. The annual incidence of KD reported from 47 prefectures in Japan was correlated strongly with the total fertility rate (TFR) recorded 15 years previously (a). In a prefecture where KD incidence was high, the TFR was low 15 years previously (b). Ref. [49] was modified.
(EPS)

S5 Fig. Birthweights and gestational ages in neonatal intensive care unit of Fukuoka Tokushukai Hospital. The mean values for birthweights and gestational ages in neonates who were admitted to the neonatal intensive care unit (NICU) increased. This increase was due to a change in NICU policy.
(EPS)

S1 Table. Linear regression coefficients to explain normalized IgG in different age groups.
(DOCX)

S2 Table. Linear regression coefficients to explain IgG in four age groups.
(DOCX)

S3 Table. Regression analysis to explain normalized IgE in different age groups.
(DOCX)

S4 Table. Regression coefficients which explain IgG, after excluding Kawasaki disease cases.
(DOCX)

Acknowledgments

Funding sources
We are grateful to Teppei Nagai, Kazuma Shibata, Akihito Hori, Shinichiro Yamauchi and Hiroshi Manabe for their assistance in data preparation and statistical analysis. There was no funding source.
Author Contributions

Conceptualization: Hiromi Yamaguchi, Yoshiro Nagao.
Data curation: Hiromi Yamaguchi.
Formal analysis: Yoshiro Nagao.
Investigation: Masaaki Hirata, Ichiro Yamane, Hisashi Endo, Hiroe Okubo.
Methodology: Kuniya Hatakeyama, Yoshimi Nishimura.
Resources: Masaaki Hirata.
Supervision: Masaaki Hirata.
Validation: Kuniya Hatakeyama.
Writing – original draft: Hiromi Yamaguchi, Yoshiro Nagao.
Writing – review & editing: Masaaki Hirata, Kuniya Hatakeyama, Ichiro Yamane, Hisashi Endo, Hiroe Okubo, Yoshimi Nishimura.

References

1. Karako K, Song P, Chen Y, Tang W, Kokudo N. Overview of the characteristics of and responses to the three waves of COVID-19 in Japan during 2020–2021. Biosci Trends. 2021; 15:1–8. Epub 20210129. https://doi.org/10.5582/bst.2021.01019 PMID: 33518668.

2. Japan Research Center/YouGov. Uses of face masks in public places: surveys in 23 countries/regions [In Japanese] 2021 [cited 2022 August 4].

3. Finlay BB, Amato KR, Azad M, Blaser MJ, Bosch TCG, Chu H, et al. The hygiene hypothesis, the COVID pandemic, and consequences for the human microbiome. Proc Natl Acad Sci U S A. 2021;118. https://doi.org/10.1073/pnas.2012171118 PMID: 33472859; PubMed Central PMCID: PMC8017729.

4. Strachan DP. Hay fever, hygiene, and household size. BMJ. 1989; 299:1259–1260. https://doi.org/10.1136/bmj.299.6710.1259 PMID: 2513902; PubMed Central PMCID: PMC1838109.

5. López-Serrano P, Pérez-Calle JL, Pérez-Fernández MT, Fernández-Font JM, Boixeda de Miguel D, Fernández-Rodríguez CM. Environmental risk factors in inflammatory bowel diseases. Investigating the hygiene hypothesis: a Spanish case-control study. Scand J Gastroenterol. 2010; 45:1464–1471. Epub 20100812. https://doi.org/10.3109/00365521.2010.510575 PMID: 20704469.

6. Bloomfield SF, Rook GA, Scott EA, Shanahan F, Stanwell-Smith R, Turner P. Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. Perspect Public Health. 2016; 136:213–224. https://doi.org/10.1177/1757913916650225 PMID: 27354505; PubMed Central PMCID: PMC4966430.

7. Seiskari T, Kondrashova A, Viskari H, Kaila M, Haapala AM, Aittoniemi J, et al. Allergic sensitization and microbial load—a comparison between Finland and Russian Karelia. Clin Exp Immunol. 2007; 148:47–52. https://doi.org/10.1111/j.1365-2249.2007.03333.x PMID: 17302731; PubMed Central PMCID: PMC1868862.

8. Snel J, Vissers YM, Smit BA, Jongen JM, van der Meulen ET, Zwijsen R, et al. Strain-specific immuno-modulatory effects of Lactobacillus plantarum strains on birch-pollen-allergic subjects out of season. Clin Exp Allergy. 2011; 41:232–242. Epub 20101201. https://doi.org/10.1111/j.1365-2222.2010.03650.x PMID: 21121978.

9. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. Epidemiology. 2012; 23:402–414. https://doi.org/10.1097/EDE.0b013e31824d5da2 PMID: 22441545.

10. Rujeni N, Naush N, Bourke CD, Midzi N, Mduluza T, Taylor DW, et al. Atopy is inversely related to schistosoma infection intensity: a comparative study in Zimbabwean villages with distinct levels of Schistosoma haematobium infection. Int Arch Allergy Immunol. 2012; 158:288–298. Epub 20120306. https://doi.org/10.1159/000332949 PMID: 22399631; PubMed Central PMCID: PMC3398828.

11. Cahenzi J, Köllner Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe. 2013; 14:559–570. https://doi.org/10.1016/j.chom.2013.10.004 PMID: 24237701; PubMed Central PMCID: PMC4049278.
12. West CE, Rydén P, Lundin D, Engstrand L, Tulic MK, Prescott SL. Gut microbiome and innate immune response patterns in IgE-associated eczema. Clin Exp Allergy. 2015; 45:1419–1429. https://doi.org/10.1111/cea.12566. PMID: 25944283.

13. Korhonen L, Oikarinen S, Lehtonen J, Mustonen N, Tyyni I, Niemelä O, et al. Rhinoviruses in infancy and risk of immunoglobulin E sensitization. J Med Virol. 2019; 91:1470–1478. Epub 20190325. https://doi.org/10.1002/jmv.25455. PMID: 30866076.

14. Mustonen N, Siljander H, Niemelä O, Ilonen J, Haahtela T, Knip M. Allergy-Related Symptoms Are Poorly Predicted by IgE and Skin Prick Testing in Early Life. Int Arch Allergy Immunol. 2021; 182:574–584. Epub 20210205. https://doi.org/10.1159/000512109. PMID: 33550294.

15. Devalapalli AP, Lesher A, Shieh K, Solow JS, Everett ML, Edala AS, et al. Increased levels of IgE and autoreactive, polyreactive IgG in wild rodents: implications for the hygiene hypothesis. Scand J Immunol. 2006; 64:125–136. Epub 2006/07/27. https://doi.org/10.1111/j.1365-3083.2006.01785.x. PMID: 16867157.

16. Flies AS, Mansfield LS, Grant CK, Weldele ML, Holekamp KE. Markedly Elevated Antibody Responses in Wild versus Captive Spotted Hyenas Show that Environmental and Ecological Factors Are Important Modulators of Immunity. PLoS One. 2015; 10:e0137679. Epub 2015/10/09. https://doi.org/10.1371/journal.pone.0137679. PMID: 26448676; PubMed Central PMCID: PMC4621877.

17. Burgner D, Carter K, Webster R, Kuijpers TW. Kawasaki disease, childhood allergy and the hygiene hypothesis. Pediatr Allergy Immunol. 2011; 22:751. Epub 2011/09/29. https://doi.org/10.1111/j.1399-3038.2011.01184.x. PMID: 21950681.

18. Fujiwara T, Shobugawa Y, Matsumoto K, Kawachi I. Association of early social environment with the onset of pediatric Kawasaki disease. Ann Epidemiol. 2019; 29:74–80. Epub 2018/11/22. https://doi.org/10.1016/annepidem.2018.10.010. PMID: 30459020.

19. Lee JK. Hygiene Hypothesis as the Etiology of Kawasaki Disease: Dysregulation of Early B Cell Development. Int J Mol Sci. 2021; 22. Epub 2021/11/28. https://doi.org/10.3390/ijms222212334. PMID: 34830213; PubMed Central PMCID: PMC8622879.

20. Tokuda M, Aoki Y, Abe H, Ohtsuka H, Ishikawa Y, Ishikawa Y, et al. Kawasaki disease in Kawasaki City: A Prospective, Population-based Study of Early B Cell Development. PLoS Negl Trop Dis. 2009; 3:e534. Epub 2009/01/23. https://doi.org/10.1371/journal.pntd.0000534. PMID: 19184962; PubMed Central PMCID: PMC2699976.

21. Chiba N, Iwatsuru K, Takahashi H, et al. Kawasaki Disease in Children of Kyiv. Analysis of 23 cases [In Ukrainian]. Sovremennaya Pediatriya. 2018; 89:116–123. https://doi.org/10.15574/SP.2018.89.116.

22. Melonari P, Abate H, Llano López LH, Cuttica RJ, Apaz MT, Battagliotti C, et al. Clinical-epidemiological characteristics and predictors of coronary complications in children with Kawasaki disease [In Spanish]. Rev Chilena Infectol. 2019; 36:636–641. https://doi.org/10.4067/s0716-10182019000500636. PMID: 31839895.

23. Tanaka N, Sekimoto K, Naoe S. Kawasaki disease. Relationship with infantile periarteritis nodosa. Arch Pathol Lab Med. 1976; 100:81–86. PMID: 3150.

24. Niimura I, Maki T. Sudden cardiac death in childhood. Jpn Circ J. 1989; 53:1571–1580. https://doi.org/10.1253/jcj.53.1571. PMID: 2638282.

25. Ogata S, Tremoulet AH, Sato Y, Ueda K, Shimizu C, Sun X, et al. Coronary artery outcomes among children with Kawasaki disease in the United States and Japan. J Int Cardiol. 2013; 168:3825–3828. Epub 2013/07/16. https://doi.org/10.1016/j.jcird.2013.06.027. PMID: 23849668; PubMed Central PMCID: PMC4002741.

26. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children [In Japanese]. Arerugi. 1967; 16:178–222.

27. Nagao Y, Urabe C, Nakamura H, Hatano N. Predicting the characteristics of the aetiologic agent for Kawasaki disease from other paediatric infectious diseases in Japan. Epidemiol Infect. 2016; 144:478–492. Erratum in p. 493.

28. Makino N, Nakamura Y, Yashiro M, Kosami K, Matsubara Y, Ae R, et al. Nationwide epidemiologic survey of Kawasaki disease in Japan, 2015–2016. Pediatr Int. 2019; 61:397–403. Epub 20190416. https://doi.org/10.1111/ped.13809. PMID: 30786119.

29. Sano T, Makino N, Aoyama Y, Ae R, Kojo T, Kotani K, et al. Temporal and geographical clustering of Kawasaki disease in Japan: 2007–2012. Pediatrics international: official journal of the Japan Pediatric Society. 2016; 58:1140–1145. Epub 2016/03/05. https://doi.org/10.1111/ped.12970. PMID: 26940079.

30. Burns JC, Cayan DR, Tong G, Bainto EV, Turner CL, Shike H, et al. Seasonality and temporal clustering of Kawasaki syndrome. Epidemiology. 2005; 16:220–225. Epub 2005/02/11. https://doi.org/10.1097/01.ede.0000152901.06689.d4. PMID: 15703537; PubMed Central PMCID: PMC2884624.
31. Nakamura Y, Yanagawa I, Kawasaki T. Temporal and geographical clustering of Kawasaki disease in Japan. Prog Clin Biol Res. 1987; 250:19–32. Epub 1987/01/01. PMID: 3423038.
32. Kuo HC, Pan CT, Huang YH, Huang FC, Lin YS, Li SC, et al. Global Investigation of Immune Repertoire Suggests Kawasaki Disease Has Infectious Cause. Circ J. 2019; 83:2070–2078. Epub 2019/08/06. https://doi.org/10.1253/circj.CJ-19-0206 PMID: 31378745.
33. Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, et al. A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat Genet. 2012; 44:517–521. Epub 20120325. https://doi.org/10.1038/ng.2220 PMID: 22446962.
34. Lee YC, Kuo HC, Chang JS, Chang LY, Huang LM, Chen MR, et al. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. Nat Genet. 2012; 44:522–525. Epub 20120325. https://doi.org/10.1038/ng.2227 PMID: 22446961.
35. Khor CC, Davila S, Breunis WB, Lee YC, Shimizu C, Wright VJ, et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat Genet. 2011; 43:1241–1246. Epub 20111113. https://doi.org/10.1038/ng.981 PMID: 22081228.
36. Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M, et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet. 2008; 40:35–42. Epub 20071216. https://doi.org/10.1038/ng.2007.59 PMID: 18084290; PubMed Central PMCID: PMC2876982.
37. Burgner D, Harnden A. Kawasaki disease: what is the epidemiology telling us about the etiology? Int J Infect Dis. 2005; 9:185–194. Epub 2005/06/07. https://doi.org/10.1016/j.ijid.2005.03.002 PMID: 15936970.
38. Google. COVID-19 Community mobility report: Google; 2022 [cited 2022 March 31]. Available from: https://google.com/covid19/mobility/.
39. Box GEP, Cox DR. An analysis of transformations. Journal of the Royal Statistical Society, Series B. 1964; 26:211–243.
40. Lam HCY, Jarvis D. Seasonal variation in total and pollen-specific immunoglobulin E levels in the European Community Respiratory Health Survey. Clin Exp Allergy. 2021; 51:1085–1088. Epub 20210515. https://doi.org/10.1111/cea.13895 PMID: 33960529.
41. Sposato B, Scalese M, Rogliani P. Seasonal monitoring of serum IgE and blood eosinophil variability may lead to a better severe asthma phenotyping and to a correct biologic prescription. J Biol Regul Homeost Agents. 2020; 34:315–318. https://doi.org/10.23812/19-429-L-49 PMID: 32538068.
42. Ballas ZK. Structure of immunoglobulins: UpToDate; 2020 [cited 2022 March 31]. Available from: https://uptodate.com.
43. Simister NE. Placental transport of immunoglobulin G. Vaccine. 2003; 24:3365. https://doi.org/10.1016/s0264-410x(03)00334-7 PMID: 12850341
44. Clements T, Rice TF, Vamvakas G, Barnett S, Barnes M, Donaldson B, et al. Update on Transplacental Transfer of IgG Subclasses: Impact of Maternal and Fetal Factors. Front Immunol. 2020; 11:1920. Epub 2020/10/06. https://doi.org/10.3389/fimmu.2020.01920 PMID: 33013843; PubMed Central PMCID: PMC7516031.
45. Japan Hygiene Products Industry Association. Japan’s production and importation of face masks (2012–2021) [In Japanese]. 2022 [cited 2022 August 2]. Available from: https://www.jhpia.or.jp/data/data7.html.
46. Ae R, Shibata Y, Kosami K, Nakamura Y, Hamada H. Kawasaki Disease and Pediatric Infectious Diseases During the Coronavirus Disease 2019 Pandemic. J Pediatr. 2021; 239:50–58.e52. Epub 20210726. https://doi.org/10.1016/j.jpeds.2021.07.053 PMID: 34324881; PubMed Central PMCID: PMC8591269.
47. Nakamura Y, Yashiro M, Uehara R, Sadakane A, Tsuboi S, Aoyama Y, et al. Epidemiologic features of Kawasaki disease in Japan: results of the 2009–2010 nationwide survey. J Epidemiol. 2012; 22:216–221. Epub 2012/03/27. https://doi.org/10.2188/jea.je201201026 PMID: 22447211.
48. Ae R, Makino N, Kosami K, Kuwabara M, Matsubara Y, Nakamura Y. Epidemiology, Treatments, and Cardiac Complications in Patients with Kawasaki Disease: The Nationwide Survey in Japan, 2017–2018. J Pediatr. 2020; 225:23–29.e22. Epub 20200523. https://doi.org/10.1016/j.jpeds.2020.05.034 PMID: 32454114.
49. Nagao Y. Decreasing fertility rate correlates with the chronological increase and geographical variation in incidence of Kawasaki disease in Japan. PLoS ONE. 2013; 8:e67934. https://doi.org/10.1371/journal.pone.0067934 PMID: 23861836
50. Yamazaki-Nakashimada MA, Gámiz-González LB, Murata C, Honda T, Yasukawa K, Hamada H. IgG levels in Kawasaki disease and its association with clinical outcomes. Clin Rheumatol. 2019; 38:749–754. Epub 2018/10/22. https://doi.org/10.1007/s10067-018-4339-0 PMID: 30343342.
51. Burns JC, Franco A. The immunomodulatory effects of intravenous immunoglobulin therapy in Kawasaki disease. Expert Rev Clin Immunol. 2015; 11:819–825. Epub 2015/06/24. https://doi.org/10.1586/1744666X.2015.1044980 PMID: 26099344; PubMed Central PMCID: PMC4985263.

52. Yanagimoto K, Nomura Y, Masuda K, Hirabayashi M, Morita Y, Yoshishige M, et al. Immunoglobulin G values before treatment are correlated with the responsiveness to initial intravenous immunoglobulin therapy for Kawasaki disease. Int Arch Allergy Immunol. 2014; 164:83–88. Epub 2014/06/07. https://doi.org/10.1159/000363383 PMID: 24903098.

53. Goto R, Inuzuka R, Shindo T, Namai Y, Oda Y, Harita Y, et al. Relationship between post-IVIG IgG levels and clinical outcomes in Kawasaki disease patients: new insight into the mechanism of action of IVIG. Clin Rheumatol. 2020; 39:3747–3755. Epub 2020/05/28. https://doi.org/10.1007/s10067-020-05153-w PMID: 32458247.

54. Morikawa Y, Ohashi Y, Harada K, Asai T, Okawa S, Nagashima M, et al. Coronary risks after high-dose gamma-globulin in children with Kawasaki disease. Pediatr Int. 2000; 42:464–469. https://doi.org/10.1046/j.1442-200x.2000.01288.x PMID: 11059532.

55. Sawaji Y, Haneda N, Yamauchi S, Kajino Y, Kishida K, Seto S, et al. Coronary risk factors in acute Kawasaki disease: correlation of serum immunoglobulin levels with coronary complications. Acta Paediatr Jpn. 1998; 40:218–225. https://doi.org/10.1111/j.1442-200x.1998.tb01915.x PMID: 9695293.

56. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. N Engl J Med. 1991; 324:1633–1639. https://doi.org/10.1056/NEJM199106063242305 PMID: 1709446.

57. Takeuchi A, Namba T, Matsumoto N, Tamai K, Nakamura K, Nakamura M, et al. Preterm birth and Kawasaki disease: a nationwide Japanese population-based study. Pediatr Res. 2021. Epub 20211008. https://doi.org/10.1038/s41390-021-01780-4 PMID: 34625654.

58. Katsunuma N, Hara N, Toda T, Sunaga Y, Yoshizawa M, Kono Y, et al. Prevention Measures for COVID-19 and Changes in Kawasaki Disease Incidence. J Epidemiol. 2021; 31:573–580. Epub 20210917. https://doi.org/10.2188/jea.JE20210132 PMID: 34483151; PubMed Central PMCID: PMCP8502831.

59. Nagao Y, Koelle K. Decreases in dengue transmission may act to increase the incidence of dengue hemorrhagic fever. Proc Natl Acad Sci U S A. 2008; 105:2238–2243. https://doi.org/10.1073/pnas.0709029105 PMID: 18250338.

60. Rock GA, Martinelli R, Brunet LR. Innate immune responses to mycobacteria and the downregulation of atopic responses. Curr Opin Allergy Clin Immunol. 2003; 3:337–342. https://doi.org/10.1097/00130832-200310000-00003 PMID: 14501431.

61. Scudellari M. News Feature: Cleaning up the hygiene hypothesis. Proc Natl Acad Sci U S A. 2017; 114:1433–1436. https://doi.org/10.1073/pnas.1700681114 PMID: 28196925; PubMed Central PMCID: PMCP5329062.

62. Box GEP, Jenkins GM, Reinsel GC. Time series analysis: forecasting and control. New Jersey: Wiley; 2008.

63. Verdoni L, Mascia M, Gervasoni A, Martelli L, Ruggeri M, Ciuflifreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020; 395:1771–1778. Epub 20200513. https://doi.org/10.1016/S0140-6736(20)31193-X PMID: 32407660; PubMed Central PMCID: PMC7220177.

64. Scudellari M. News Feature: Cleaning up the hygiene hypothesis. Proc Natl Acad Sci U S A. 2017; 114:1433–1436. https://doi.org/10.1073/pnas.1700681114 PMID: 28196925; PubMed Central PMCID: PMCP5329062.

65. Box GEP, Jenkins GM, Reinsel GC. Time series analysis: forecasting and control. New Jersey: Wiley; 2008.

66. Verdoni L, Mascia M, Gervasoni A, Martelli L, Ruggeri M, Ciuflifreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020; 395:1771–1778. Epub 20200513. https://doi.org/10.1016/S0140-6736(20)31193-X PMID: 32407660; PubMed Central PMCID: PMC7220177.

67. Scudellari M. News Feature: Cleaning up the hygiene hypothesis. Proc Natl Acad Sci U S A. 2017; 114:1433–1436. https://doi.org/10.1073/pnas.1700681114 PMID: 28196925; PubMed Central PMCID: PMCP5329062.

68. Box GEP, Jenkins GM, Reinsel GC. Time series analysis: forecasting and control. New Jersey: Wiley; 2008.

69. Verdoni L, Mascia M, Gervasoni A, Martelli L, Ruggeri M, Ciuflifreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020; 395:1771–1778. Epub 20200513. https://doi.org/10.1016/S0140-6736(20)31193-X PMID: 32407660; PubMed Central PMCID: PMC7220177.

66. Nagao Y. Kawasaki Disease Integrated Information Sharing System (Kawa-iiss). The 13th International Kawasaki Disease Symposium (Online) 2021.