INFECTION-RELATED IMMUNE-MEDIATED DISEASES AND MICROBIOTA

EDITED BY: Kyung-Yil Lee, Hiromichi Hamada and Miika Kaleva Arvonen
PUBLISHED IN: Frontiers in Pediatrics

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-744-7
DOI 10.3389/978-2-88963-744-7

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

INFECTION-RELATED IMMUNE-MEDIATED DISEASES AND MICROBIOTA

Topic Editors:
Kyung-Yil Lee, The Catholic University of Korea, South Korea
Hiromichi Hamada, Tokyo Women's Medical University Yachiyo Medical Center, Japan
Miika Kaleva Arvonen, Department of Pediatrics, Kuopio University Hospital, Finland

Citation: Lee, K.-Y., Hamada, H., Arvonen, M. K., eds. (2020). Infection-Related Immune-Mediated Diseases and Microbiota. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-744-7

Table of Contents

frontiers in Pediatrics

EDITORIAL
published: 07 April 2020
doi: 10.3389/fped.2020.00108

Editorial: Infection-Related Immune-Mediated Diseases and Microbiota

Kyung-Yil Lee[1]*, *Hiromichi Hamada*[2] and *Miika Arvonen*[3]

[1] The Catholic University of Korea, College of Medicine, Seoul, South Korea, [2] Department of Pediatrics, Tokyo Women's Medical University Yachiyo Medical Center, Chiba, Japan, [3] Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland

Keywords: microbiota, Kawasaki disease, juvenile idiopathic arthritis, Hirschsprung's disease, etiology, epidemiology, pathogenesis, multiple-drug resistant Staphylococcus aureus

Editorial on the Research Topic

Infection-Related Immune-Mediated Diseases and Microbiota

Microbiota may form a collaborative partnership with the host. Eubiosis, i.e., the homeostatic state of the host and symbiotic microflora, is critical for maintaining a state of well-being in the host. Commensal bacteria may prevent by colonization by external pathogens, and the mucosal immune system, such as gut-associated lymphoid tissues (GALT), is established after colonization of the gut by normal flora, suggesting that microbiota and host immune systems are closely related to each other (1). On the other hand, certain strains of microbes can elicit infectious events on occasion when they invade into the host and induce an immune reaction.

The microbiota in an individual continuously changes after birth (2), and certain strains of microbes in ethnic groups may be influenced by environmental factors such as diet and socio-economic state. Thus, the microbiota is different in different populations and can be changed by the changing environment. The disruption of the reciprocal equilibrium between microbiota and host, that is, dysbiosis, has now become a major subject of study in various medical fields, including those of metabolic, gastroenteric, psychiatric, neurologic, and allergic diseases and cancers. Also, intestinal dysbiosis has been reported in patients with Kawasaki disease (KD) and juvenile idiopathic arthritis (JIA) (3, 4). Although the mechanisms relating dysbiosis and provocation of diseases remain elusive, over-production of toxic materials from dysbiosis-causing microbials, vulnerable invasion of microbials through wakened mucosal barriers, or the disruption of the homeostatic relationship between the microbiota and the host's immune system may be responsible for disease onset [(5), Esposito et al.].

The human gut microbiota is composed of over 1,000 species, and different strains are colonized in the small intestine and colon. Hirschsprung's disease (HD) is characterized by a defect of intestinal nerve ganglia, and occasionally patients with HD can be affected with HD-associated enterocolitis before or after operation. In the majority of cases, the causative agents of HD-associated enterocolitis are not external pathogens, and dysbiosis may be associated with intestinal inflammation (6). A different microbiome between patients with total colon resection and those with partial resection is observed, and the former group tends to have a higher risk of enterocolitis (Pini Prato et al.). Staphylococcus species are one of the main normal flora that colonize the skin and, on occasion, the vagina tract of pregnant women. Normal flora in neonates begin to colonize just after birth, though some strains may be different according to delivery method (Caesarian section or vaginal delivery). Although colonization by commensals, including multiple-drug-resistant *S. aureus*, may be a risk factor for subsequent infection, severe invasive infection is

Edited by:
Marzia Duse,
Sapienza University of Rome, Italy

Reviewed by:
Riccardo Castagnoli,
University of Pavia, Italy

***Correspondence:**
Kyung-Yil Lee
leekyungyil@catholic.ac.kr

Specialty section:
This article was submitted to Pediatric Immunology, a section of the journal Frontiers in Pediatrics

Received: *04 October 2019*
Accepted: *02 March 2020*
Published: *07 April 2020*

Citation:
Lee K-Y, Hamada H and Arvonen M (2020) Editorial: Infection-Related Immune-Mediated Diseases and Microbiota. Front. Pediatr. 8:108. doi: 10.3389/fped.2020.00108

rare in immune-competent hosts. It is observed that colonized multiple-drug-resistant *S. aureus* in neonates could be transmitted from their mothers (Lin and Yao).

Childhood immune-mediated diseases, including KD and JIA, may be associated with prevalent infections in childhood. Interestingly, the incidence of both diseases is quite different among populations; the incidence of KD is over 10–20 times higher in East Asian countries such as Japan and Korea, and the incidence of JIA is over 10 times higher in North European countries compared to children in East Asian countries (7). The discrepancy of incidence rates across the populations has been observed in other infection-related immune-mediated diseases, including type I diabetes, inflammatory bowel disease, and Behcet disease. Although genetic or environmental factors may be responsible for the finding, it is possible that children living in higher-prevalence countries may have more chances of being exposed to KD or JIA pathogens, since the clinical manifestations and immune function of children with KD or JIA are nearly identical across the populations.

Based on the epidemiological and clinical characteristics of KD, it has been suggested that it may be associated with infectious agents, including viruses, especially RNA viruses (Rowley and Schulman), bacteria that can activate innate and adaptive immune systems (Nagata; Nakamura et al.), and strains of normal flora (Esposito et al.; Rhim et al.). Since KD is a self-limiting systemic inflammation, many laboratory parameters are affected during the natural course of the disease; levels of white blood cells, erythrocyte sedimentation rate, C-reactive protein, albumin, hemoglobin, and other biomarkers such as proinflammatory cytokines are up-regulated or down-regulated, and various genetic traits are related to KD susceptibility or phenotype (Chaudhary et al.). Intravenous immunoglobulin (IVIG) treatment is known to reduce the risk of coronary artery lesions (CALs), a major complication of KD. It was reported that a patient group with spontaneous defervescence had a higher rate of CALs (aneurysms) at 1 month after disease onset compared to the IVIG-treated group (Hu et al.). However, a small proportion of initially CAL-negative patients in both groups showed CALs at 1 month, suggesting that some KD patients may have ongoing inflammation in CALs after defervescence (8).

There are few human diseases of which the pathogenesis has been clearly proven from the era of Hippocrates to the present time. Although etiologic agents have been discovered in infectious diseases, the substances inducing inflammation and tissue cell injury in infectious diseases and infection-related immune-mediated diseases are not pathogens themselves but smaller substances derived from the infectious insults (9). Many researchers may agree on the notions that every disease has etiologic substances and that present immunological concepts have limitations in explaining the pathogenesis of many diseases. It is now known that the host immune system reacts not to only the substances derived from the infectious agents, including toxins and pathogen-associated molecular patterns (PAMPs), but also to the substances derived from host cells injured by infectious insults such as damage (danger)-associated molecular patterns (DAMPs), especially in cases of intracellular infection such as viral or intracellular pathogen infections (10). Because of the appearance of KD as a novel disease in East Asia, the discovery of the etiology and pathogenesis of KD will help to extend our understanding regarding these issues of human diseases. The pathogenesis of KD is associated with the immune reaction of the host against infectious insults. The contributions to this Research Topic present and discuss various aspects of the pathogenesis of KD, including the roles of components of the adaptive immune system such as IgA plasma cells and cytotoxic CD8 T cells against viral antigens (Rowley and Schulman), a similar/common immune process associated with the activation of T cells and innate immune cells caused by diverse pathogens (Nagata), an innate immune response including PAMPs, toll-like receptors, and complement pathways (Nakamura et al.), and types of DAMPs produced after infectious insults based on the protein-homeostasis-system hypothesis (Rhim et al.).

AUTHOR CONTRIBUTIONS

K-YL wrote the manuscript. HH and MA have made a substantial, direct, and intellectual contribution to the work. All authors approved it for publication.

REFERENCES

1. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature.* (2012) 489:231–41. doi: 10.1038/nature11551
2. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA.* (2011) 108:4578–85. doi: 10.1073/pnas.1000081107
3. Arvonen M, Berntson L, Pokka T, Karttunen TJ, Vähäsalo P, Stoll ML. Gut microbiota-host interactions and juvenile idiopathic arthritis. *Pediatr Rheumatol Online J.* (2016) 14:44. doi: 10.1186/s12969-016-0104-6
4. Kinumaki A, Sekizuka T, Hamada H, Kato K, Yamashita A, Kuroda M. Characterization of the gut microbiota of Kawasaki disease patients by metagenomic analysis. *Front Microbiol.* (2015) 6:824. doi: 10.3389/fmicb.2015.00824
5. Milshteyn A, Colosimo DA, Brady SF. Accessing bioactive natural products from the human microbiome. *Cell Host Microbe.* (2018) 23:725–36. doi: 10.1016/j.chom.2018.05.013
6. Gosain A, Brinkman AS. Hirschsprung's associated enterocolitis. *Curr Opin Pediatr.* (2015) 27:364–9. doi: 10.1097/MOP.0000000000000210
7. Thierry S, Fautrel B, Lemelle I, Guillemin F. Prevalence and incidence of juvenile idiopathic arthritis: a systematic review. *Joint Bone Spine.* (2014) 81:112–7. doi: 10.1016/j.jbspin.2013.09.003
8. Seo YM, Kang HM, Lee SC, Yu JW, Kil HR, Rhim JW, et al. Clinical implications in laboratory parameter values in acute Kawasaki disease for early diagnosis and proper treatment. *Korean J Pediatr.* (2018) 61:160–6. doi: 10.3345/kjp.2018.61.5.160

9. Lee KY. A common immunopathogenesis mechanism for infectious diseases: the protein-homeostasis-system hypothesis. *Infect Chemother.* (2015) 47:12–26. doi: 10.3947/ic.2015.47.1.12
10. Matzinger P. The danger model: a renewed sense of self. *Science.* (2002) 296:301–5. doi: 10.1126/science.1071059

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

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 05 December 2018
doi: 10.3389/fped.2018.00384

Maternal-Infant Correlation of Multidrug-Resistant *Staphylococcus aureus* Carriage: A Prospective Cohort Study

*Jialing Lin and Zhenjiang Yao**

Department of Epidemiology and Health Statistics, School of Public Health, Guangdong Pharmaceutical University, Guangzhou, China

Objectives: We aim to assess the correlation of multidrug-resistant *Staphylococcus aureus* (MDR *S. aureus*) carriage between mothers and their newborn infants.

Materials and Methods: We conducted a prospective cohort study of mothers and their newborn infants in two hospitals in Shenzhen, China, from August to November 2015. We collected demographic and clinical information from mothers and newborn infants by face-to-face questionnaires and medical datasets. Serial swabs were collected from mothers and their newborn infants for further experiments. Maternal-infant correlation was assessed using the Poisson regression model.

Results: The prevalence of MDR *S. aureus* vaginal carriage in mothers was 4.7% (86/1834). The incidence of MDR *S. aureus* carriage in newborn infants was 1.3% (23/1834). The adjusted relative risk and 95% confidence interval of maternal-infant MDR *S. aureus* carriage was 7.63 (2.99–19.49). Six MDR *S. aureus* maternal-infant pairs were concordant. The phenotypic and molecular characteristics of MDR *S. aureus* isolates were similar between mothers and their newborn infants.

Conclusion: MDR *S. aureus* vaginal carriage in mothers was associated with an increased risk for MDR *S. aureus* carriage in their newborn infants.

Keywords: *Staphylococcus aureus*, multidrug resistant, mothers, infants, cohort

Edited by:
Hiromichi Hamada, Tokyo Women's Medical University Yachiyo Medical Center, Japan

Reviewed by:
Marine Butin, Centre Hospitalier Universitaire de Lyon, France
Guillermo Soza, Universidad de La Frontera, Chile
Hiroyuki Shiro, Yokohama Rosai Hospital, Japan

****Correspondence:***
Zhenjiang Yao
zhjyao2001@yahoo.com

Specialty section:
This article was submitted to Pediatric Infectious Diseases, a section of the journal Frontiers in Pediatrics

Received: *21 September 2018*
Accepted: *20 November 2018*
Published: *05 December 2018*

Citation:
Lin J and Yao Z (2018) Maternal-Infant Correlation of Multidrug-Resistant Staphylococcus aureus Carriage: A Prospective Cohort Study. Front. Pediatr. 6:384. doi: 10.3389/fped.2018.00384

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most important human bacterial pathogens that cause nosocomial and community infections, and results in substantial morbidity and mortality (1). Multidrug-resistant *S. aureus* (MDR *S. aureus*) is of greater significance because of its more severe clinical outcomes (2).

Those persons who are colonized with MDR *S. aureus* have a higher risk for subsequent infections than non-colonized individuals (3, 4). Most infections are caused by the same MDR *S. aureus* strain that previously colonized the person (5). In response to these findings, there has been increasing attention to the detection of MDR *S. aureus* carriage, with subsequent decolonization of carriers as a potential method for the prevention of MDR *S. aureus* infection.

Most of the available data on the detection of MDR *S. aureus* carriage have been in hospitalized non-pregnant adults (6, 7). Despite the fact that MDR *S. aureus* outbreaks in infants have been

linked to the infected or colonized mother, there are limited data on MDR *S. aureus* carriage rates among mothers or on the risk of transmission of MDR *S. aureus* from pregnant MDR *S. aureus* carriers to their newborn infants (8, 9). The maternal-infant relatedness of MDR *S. aureus* carriage is of particular interest.

Accordingly, we determined whether vaginal carriage of MDR *S. aureus* in the mothers is independently correlated with MDR *S. aureus* carriage in their newborn infants. Antimicrobial susceptibility, virulence factors, and clonality of isolates were also evaluated. The hypothesis of this study is that there is maternal-infant vertical MDR *S. aureus* carriage.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Guangdong Pharmaceutical University and was performed in accordance with the approved guidelines. Written informed consent was obtained from the mothers and their newborn infants involved in the study before enrollment.

This prospective cohort study was conducted in two hospitals in Shenzhen, China, between August and November 2015. The two hospitals, Longhua Central Hospital and Guanlan People's Hospital, are large hospitals whose obstetric services deliver between 10,000 and 15,000 neonates per year; these are two of the largest delivery hospitals in China. The target populations were mothers and their newborn infants. Chinese mothers with gestation between 35 and 40 weeks were voluntarily included. Mothers with twin or multiple gestations, cesarean section, or acute diseases were excluded. Newborn infants without sample collection were also excluded. The sample size was calculated using the power two proportions method with 4.00% proportion (approximate prevalence of *S. aureus* in the newborns), 4.00 ratio, 0.05 alpha, and 0.80 power. Taking a 10% loss of follow-up into consideration, the estimated minimum sample size of this study was 107 for each group.

A face-to-face questionnaire was administered by trained personnel that aimed to collect demographic information and information regarding potential factors influencing MDR *S. aureus* carriage during pregnancy. Medical records of the mothers and their newborn infants were reviewed by two members of the study team who were blinded to the maternal and infant MDR *S. aureus* carriage status.

Sterile swabs moistened with sterile saline water were used to get specimens from the vagina of the enrolled mothers prior to delivery by trained personnel. Infant specimens for MDR *S. aureus* were sampled from the nasal cavity, ear canal, oral cavity, and umbilicus by trained nurses immediately after birth. All specimens were then inoculated into enrichment broth tubes containing 1% tryptone, 7.5% sodium chloride, 1% mannitol, and 0.25% yeast extract.

All included mothers were informed of the research question and outcome measures by well-trained staff in person before participating. The trained personnel informed the mothers of the results of *S. aureus* carriage by telephone calls. Chinese mothers with gestation between 35 and 40 weeks were voluntarily included in this study. Included mothers were asked to complete face-to-face questionnaires and collected vaginal samples by trained personnel.

Swabs were inoculated into mannitol salt agar plates at 37 ± 1°C for 24 h of incubation. Isolates were confirmed to be *S. aureus* by a combination of gram staining, catalase reaction, hemolysis test, DNase test, and coagulase tests.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines of 2015. The following antimicrobial disks were used: cefoxitin, clindamycin, rifampicin, moxifloxacin, tobramycin, gentamicin, sulfamethoxazole-trimethoprim, linezolid, and erythromycin. Isolates were defined as resistant if they were resistant or intermediate to the antimicrobial disk. *S. aureus* isolates were classified as MDR if they were resistant to no less than three antibiotic classes (10).

Multilocus sequence typing (MLST) was used to type MDR *S. aureus*, which involved sequencing the seven housekeeping genes (11). Then, the sequence types (STs) and allelic profiles were confirmed by querying the MLST database (http://eburst.mlst.net). STs were clustered into a clonal complex (CC) by using the eBURST software program (Department of Infectious Disease Epidemiology, Imperial College London, London, UK; http://eburst.mlst.net). The presence of virulence genes including Panton-valentine leukocidin (*Pvl*), toxic shock syndrome toxin (*Tst*), exfoliative toxin A (*Eta*), and exfoliative toxin B (*Etb*) using polymerase chain reaction assays as in previous studies was determined as previously described (12, 13).

All data were entered in duplicate into the EpiData version 3.0 database (The EpiData Association, Odense Denmark). Missing data were excluded. Categorical variables were compared by Pearson's chi-squared test or the Fisher exact test when appropriate. To identify variables that might confound the correlation of MDR *S. aureus* carriage between mothers and newborn infants, influencing factors with a P-value < 0.2 were identified as adjustment variables using the Poisson regression model to estimate the correlations of isolates between mothers and newborn infants. Relative risks (RRs) and 95%confidence intervals (CIs) were used to assess the maternal-infant relatedness of MDR *S. aureus* carriage. The relationship between populations and main STs of MDR *S. aureus* isolates was illustrated by a minimum spanning tree (PHYLOVIZ software version 2.0; http://www.phyloviz.net). A two-sided P-value < 0.05 was considered as being of statistical significance. All analyses were performed using Stata version 14.2 (College Station, Texas, USA).

RESULTS

Overall, 1968 mothers were preliminarily enrolled, but only 1,834 mothers and their newborn infants met the inclusion criteria and consented to participate in this study. We found that there were 133 *S. aureus* isolates and the prevalence of MDR *S. aureus* vaginal carriage in mothers was 4.7% (86/1834). There was no statistical difference of MDR *S. aureus* carriage in mothers from the two different hospitals ($\chi^2 = 0.029$, P-value $= 0.865$).

There were 60 *S. aureus* isolates and the incidence of MDR *S. aureus* carriage in newborn infants was 1.3% (23/1834). There was no statistical difference of MDR *S. aureus* carriage in newborn infants from the two different hospitals ($\chi^2 = 0.846$, P-value = 0.358).

MDR *S. aureus* vaginal carriage in mothers ($\chi^2 = 69.163$, P-value < 0.001) was significantly correlated with MDR *S. aureus* carriage in their newborn infants. However, there were no other significant variables correlated with MDR *S. aureus* carriage in infants. More details can be found in **Table 1**.

After adjusting for the age of mothers, education level of mothers, average monthly income, and gender of newborn infants, MDR *S. aureus* vaginal carriage in mothers was still a risk factor for MDR *S. aureus* carriage in newborn infants [adjusted RR (aRR) = 7.63; 95%CI, 2.99–19.49; P-value < 0.001]. More details can be found in **Table 2**.

The most predominant proportion of antibiotic resistance in the 86 MDR *S. aureus* isolates from mothers was penicillin (97.7%), followed by erythromycin (73.3%), clindamycin (64.0%), tobramycin (38.4%), cefoxitin (26.7%), trimethoprim-sulfamethoxazole (20.9%), moxifloxacin (19.8%), gentamicin (12.8%), linezolid (5.8%), and rifampicin (2.3%). The most predominant proportion of antibiotic resistance in 23 MDR *S. aureus* isolates from the newborn infants was penicillin (95.7%), followed by erythromycin (91.3%), clindamycin (69.6%), cefoxitin (47.8%), trimethoprim-sulfamethoxazole (30.4%), gentamicin (21.7%), tobramycin (21.7%), rifampicin (21.7%), moxifloxacin (13.0%), and linezolid (8.7%). The proportion of resistant rifampicin (P-value = 0.004) in MDR *S. aureus* isolates was significantly different between mothers and their newborn infants. More details can be found in **Table 3**.

The results indicated that the proportions of virulence genes of isolates in both mothers and their newborns were low. The

TABLE 2 | Correlations of multidrug-resistant *Staphylococcus aureus* carriage between mothers and their newborn infants in Shenzhen, 2015.

RR category	RR/aRR (95% CI)	z	*P*-value
Crude RR	7.17 (2.83–18.19)	4.15	<0.001
Adjusted RR[a]	7.63 (2.99–19.49)	4.24	<0.001

MDR S. aureus, multidrug-resistant Staphylococcus aureus; RR, relative risk; aRR, adjusted relative risk; CI, confidence interval.

[a] *It was adjusted for age of mothers, education level of mothers, average monthly income, and gender of newborn infants.*

TABLE 1 | Characteristics of multidrug-resistant *Staphylococcus aureus* carriage among newborn infants in Shenzhen, 2015 [n (%)].

Characteristics	Non-MDR *S. aureus* (*n* = 1811)	MDR *S. aureus* (*n* = 23)	χ^2	*P*-value
MOTHERS				
Vaginal carriage	80 (4.4)	6 (26.1)	23.862	<0.001
Age, year (>35)	137 (7.6)	2 (8.7)	NA	0.692[a]
Education (below high school)	1280 (70.7)	20 (87.0)	2.916	0.088
Average monthly income, yuan (<5000)	1013 (55.9)	17 (73.9)	2.981	0.084
Natural impregnation	1786 (98.6)	23 (100.0)	NA	1.000[a]
First pregnancy	602 (33.2)	5 (21.7)	1.357	0.244
First parturition	916 (50.6)	9 (39.1)	1.191	0.275
History of abortion	751 (41.5)	10 (43.5)	0.038	0.846
Frequency of vaginal examination after hospitalization (≤2)	947 (52.3)	13 (56.5)	2.108	0.349
Vaginitis	157 (8.7)	1 (4.4)	NA	0.715[a]
Premature rupture of membranes	8 (0.4)	0 (0.0)	NA	1.000[a]
Days of hospitalization (>3)	1380 (76.2)	16 (69.6)	0.550	0.458
Weeks of pregnancy (<37)	75 (4.1)	1 (4.4)	NA	1.000[a]
Tobacco use during pregnancy	5 (0.3)	0 (0.0)	NA	1.000[a]
Antibiotic use during pregnancy	120 (6.6)	1 (4.4)	NA	1.000[a]
Mammal pet owner	71 (3.9)	2 (8.7)	NA	0.232[a]
NEWBORN INFANTS				
Male gender	977 (54.0)	14 (60.9)	0.438	0.508
Birth weight, grams (<2500)	133 (7.3)	3 (13.0)	NA	0.240[a]
Admission to neonatology ward	158 (8.7)	1 (4.4)	NA	0.715[a]
Apgar 1st min ≤ 3	4 (0.2)	0 (0.0)	NA	NA[b]
Apgar 5th min ≤ 6	4 (0.2)	0 (0.0)	NA	NA[b]

MDR S. aureus, multidrug-resistant Staphylococcus aureus; n, number of isolates; NA, not applicable.

[a] *The P-values were calculated with the Fisher's exact test.*

[b] *No estimate of the P-value is provided owing to the lack of occurrence of the outcome of interest in at least one group.*

TABLE 3 | Phenotypic and genetic characteristics of multidrug-resistant *Staphylococcus aureus* isolates between mothers and their newborn infants in Shenzhen, 2015 [n (%)].

Characteristics	Mothers ($n = 86$)	Infants ($n = 23$)	χ^2	*P*-value
RESISTANCE PHENOTYPE (RESISTANT)				
Cefoxitin	23 (26.7)	11 (47.8)	3.758	0.053
Erythromycin	63 (73.3)	21 (91.3)	3.344	0.067
Penicillin	84 (97.7)	22 (95.7)	NA	0.513[a]
Gentamicin	11 (12.8)	5 (21.7)	NA	0.322[a]
Clindamycin	55 (64.0)	16 (69.6)	0.252	0.616
Rifampicin	2 (2.3)	5 (21.7)	NA	0.004[a]
Linezolid	5 (5.8)	2 (8.7)	NA	0.637[a]
Moxifloxacin	17 (19.8)	3 (13.0)	NA	0.558[a]
Trimethoprim-sulfamethoxazole	18 (20.9)	7 (30.4)	0.928	0.336[a]
Tobramycin	33 (38.4)	5 (21.7)	2.211	0.137
VIRULENCE GENE (POSITIVE)				
Pvl	3 (3.5)	1 (4.4)	NA	1.000[a]
Tst	2 (2.3)	0 (0.0)	NA	1.000[a]
Eta	1 (1.2)	2 (8.7)	NA	0.112[a]
Etb	0 (0.0)	0 (0.0)	NA	NA[b]

MDR S. aureus, multidrug-resistant Staphylococcus aureus; n, number of isolates; Pvl, panton-valentine leukocidin; Tst, toxic shock syndrome toxin; Eta, exfoliative toxin A; Etb, exfoliative toxin B; NA, not applicable.

[a] *The P-values were calculated with the Fisher's exact test.*

[b] *No estimate of the P-value is provided owing to the lack of occurrence of the outcome of interest in at least one group.*

proportion of the positive *Pvl* gene in MDR *S. aureus* isolates was significantly different between mothers and newborn infants (Fisher's exact test, *P*-value = 0.041). There were no significant differences of virulence genes in MDR *S. aureus* isolates between mothers and their newborn infants. More details can be found in **Table 3**.

The most common CC of 86 MDR *S. aureus* isolates in mothers was CC5 (51.2%), followed by CC7 (19.8%), CC59 (14.0%), CC88 (5.8%), CC398 (3.5%), CC20 (2.3%), CC45 (2.3%), and CC121 (1.2%). The most common CC of 23 MDR *S. aureus* isolates in newborn infants was CC5 (38.1%), followed by CC59 (28.6%), CC45 (9.5%), CC88 (9.5%), CC7 (4.8%), CC22 (4.8%), and CC398 (4.8%).

The minimum spanning tree demonstrated a good concordance of certain STs between mothers and their newborn infants; for example, MDR *S. aureus* isolates belonging to ST1, ST6, ST7, ST59, ST88, ST398, and ST965 were found in both mothers and newborn infants. More details can be found in **Figure 1**.

Overall, there were six MDR *S. aureus* maternal-infant pairs. These six MDR *S. aureus* maternal-infant pairs were concordant, with the same phenotypic and molecular characteristics. More details can be found in **Table 4**.

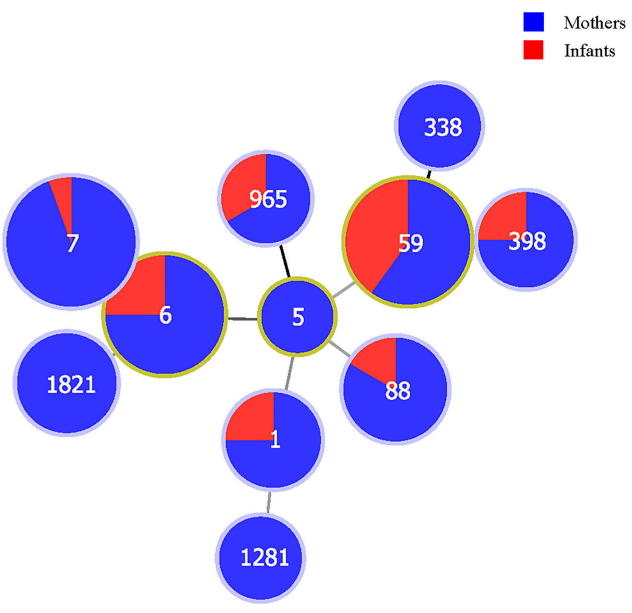

FIGURE 1 | The correlations between populations (mothers and newborn infants) and sequence types of multidrug-resistant *Staphylococcus aureus* isolates in Shenzhen, 2015. The numbers in the circles are the sequence types. The size of the circles represents the number of isolates. The distance of lines between each circle represents the degree of the relationship.

DISCUSSION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) carriage between mothers and their infants has been reported, but no previous studies reported the proportions of MDR *S. aureus* in both mothers and infants. The prevalence of MRSA in MDR *S. aureus* among mothers and their newborns was 26.7 and 47.8%,

TABLE 4 | Characteristics of six maternal-infant pairs with multidrug-resistant *Staphylococcus aureus* isolates in Shenzhen, 2015.

Pair	Population	CC	ST	Antibiotic resistance										Virulence			
				FOX	EM	PCN	GM	CM	REP	LZD	MXF	TMP/SMX	TOB	*Pvl*	*Tst*	*Eta*	*Etb*
1	Mother	5	1	−	+	+	+	−	−	−	−	+	−	−	−	−	−
	Infant	5	1	−	+	+	+	−	−	−	−	+	−	−	−	−	−
2	Mother	5	6	−	+	+	−	+	−	−	−	−	−	−	−	−	−
	Infant	5	6	−	+	+	−	+	−	−	−	−	−	−	−	−	−
3	Mother	5	188	+	+	+	−	+	−	−	+	+	−	−	−	−	−
	Infant	5	188	+	+	+	−	+	−	−	+	+	−	−	−	−	−
4	Mother	5	965	+	+	+	−	+	−	−	−	−	−	−	−	−	−
	Infant	5	965	+	+	+	−	+	−	−	−	−	−	−	−	−	−
5	Mother	59	59	+	+	+	−	+	−	−	−	−	+	−	−	−	−
	Infant	59	59	+	+	+	−	+	−	−	−	−	+	−	−	−	−
6	Mother	59	59	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	Infant	59	59	+	+	+	−	−	−	−	−	−	−	−	−	−	−

MDR S. aureus, multidrug-resistant Staphylococcus aureus; CC, clonal complex; ST, sequence type; FOX, cefoxitin; EM, erythromycin; PCN, penicillin; GM, gentamicin; CM, clindamycin; RFP, rifampicin; LZD, linezolid; MXF, moxifloxacin; TOB, tobramycin; TMP/SMX, trimethoprim-sulfamethoxazole; MDR, multidrug-resistance, resistant to no less than three antibiotic classes; Pvl, panton-valentine leukocidin; Tst, toxic shock syndrome toxin; Eta, exfoliative toxin A; Etb, exfoliative toxin B; +, positive; −, negative.

respectively. On the contrary, the prevalence of MDR *S. aureus* in MRSA among mothers and their newborns were 88.5 and 91.7%, respectively. These results were similar to current observed studies that MRSA is always MDR *S. aureus*, but MDR *S. aureus* is not necessarily MRSA (7, 11). Therefore, it is significant to assess the mother-infant relationship of MDR *S. aureus*. An American study reported that the proportion of MDR *S. aureus* carriage in community residents was 6.9% (14). Other previous studies have reported that the prevalence of *S. aureus* vaginal carriage in mothers (pregnant women) and infants ranged from 0.96–12.6% (15–18) to 5.4–17.7% (15, 16, 18–21), respectively. Therefore, the results suggested that MDR *S. aureus* carriage in our target populations were moderate when compared with other countries and regions.

This prospective cohort study identified risk factors for MDR *S. aureus* carriage in newborn infants and assessed the risk of MDR *S. aureus* vaginal carriage of mothers for MDR *S. aureus* carriage in their newborn infants. The gender of the newborn infants was not associated with MDR *S. aureus* isolates in this study, which was different to previous studies (22, 23). The mechanism between gender and MDR *S. aureus* carriage requires further exploration. No previous studies have particularly explored the maternal-infant relatedness of MDR *S. aureus* carriage. Therefore, we compared our results with other previous maternal-infant *Staphylococcus aureus* carriage. The aRR of maternal-infant MDR *S. aureus* carriage in our study was 7.63. We found that it was much higher than other previous studies, which reported that the RRs of maternal-infant *Staphylococcus aureus* carriage ranged from 2.04 to 5.70 (21, 24–26). The results demonstrated that maternal-infant MDR *S. aureus* transmission is much more hazardous.

There were few significant differences on phenotypic and virulence genetic characterizations in MDR *S. aureus* isolates between mothers and their newborn infants. We observed good consistency on certain STs of MDR *S. aureus* isolates between mothers and their newborn infants, such as ST1, ST6, ST7, ST59, ST88, ST398, and ST965. These STs were also reported in other previous studies (20, 27). Furthermore, we found that six concordant maternal-infant MDR *S. aureus* pairs had the same phenotypic and molecular characteristics. These results could further verify the homology of maternal-infant MDR *S. aureus* carriage.

In the current study, we found that more than 5% of MDR *S. aureus* isolates were resistant to linezolid and the rate was higher in isolates from newborns. This was not usual, and it could raise major issues in the case of infection with these strains (28). Healthcare workers should pay greater attention to mothers and newborns with linezolid resistance.

This study has some limitations. First, the correlation of MDR *S. aureus* carriage between mothers and infants needs to be explored further because of the limited sample size in this study. Second, whole-genome sequencing would further strengthen this study. Third, the potential importance of environmental contamination in MDR *S. aureus* carriage would need to be further explored. Fourth, participants in this study were volunteers, recruited using a convenient sampling approach. It is possible that selection bias may have occurred. Last, we did not follow up with the newborns because of insufficient financial and human support.

In summary, we identified vertical maternal-infant transmission of MDR *S. aureus* carriage in newborn infants. Newborn infants born with maternal MDR *S. aureus* carriage appear to be at higher risk of MDR *S. aureus* carriage. Accordingly, routine surveillance for MDR *S. aureus* carriage in mothers may be indicated. Prevention measures focused on controlling the spread of MDR *S. aureus* in mothers

could be a more effective strategy when outbreaks of MDR *S. aureus* in newborn infants occur. Further work should seek to elucidate the potential role of maternally derived antibodies in modifying MDR *S. aureus* carriage risk in newborn infants.

AUTHOR CONTRIBUTIONS

ZY designed the study, performed the data analysis, and revised the manuscript. JL collected the information, conducted the experiments, performed the data analysis, and wrote the manuscript.

FUNDING

This work was funded by the Innovation Fund of Guangdong Science and Technology Planning Project (grant number 2014A020213013) and Guangdong Pharmaceutical University Innovation and Strength School Funding Project (No.2016). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We greatly thank Qianting Ou and Dongxin Lin for helping us with collecting samples and conducting the experiments.

REFERENCES

1. Lowy FD. Staphylococcus aureus infections. *N Engl J Med.* (1998) 339:520–32. doi: 10.1056/NEJM199808203390806
2. Matteo B, Elda R. Multidrug-resistant bacteria: what is the threat? *Hematology Am Soc Hematol Educ Program* (2013) 2013:428–32. doi: 10.1182/asheducation-2013.1.428
3. Eells SJ, Chira S, David CG, Craft N, Miller LG. Non-suppurative cellulitis: risk factors and its association with Staphylococcus aureus colonization in an area of endemic community-associated methicillin-resistant S. aureus infections. *Epidemiol Infect.* (2011) 139:606–12. doi: 10.1017/S0950268810001408
4. Taormina DP, Konda SR, Liporace FA, Egol KA. Can preoperative nasal cultures of Staphylococcus aureus predict infectious complications or outcomes following repair of fracture nonunion? *J Infect Public Health* (2017) 839:1–5. doi: 10.1016/j.jiph.2017.10.007
5. Price JR, Cole K, Bexley A, Kostiou V, Eyre DW, Golubchik T, et al. Transmission of Staphylococcus aureus between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect. Dis.* (2017) 17:207–14. doi: 10.1016/s1473–3099(16)30413–3
6. Ungureanu A, Zlatian O, Mitroi G, Drocas A, Tirca T, Calina D, et al. Staphylococcus aureus colonisation in patients from a primary regional hospital. *Mol Med Rep.* (2017) 16:8771–80. doi: 10.3892/mmr.2017.7746
7. Tfifha M, Ferjani A, Mallouli M, Mlika N, Abroug S, Boukadida J. Carriage of multidrug-resistant bacteria among pediatric patients before and during their hospitalization in a tertiary pediatric unit in Tunisia. *Libyan J Med.* (2018) 13:1419047. doi: 10.1080/19932820.2017.1419047
8. Williams K, Hopkins S, Turbitt D, Seng C, Cookson B, Patel BC, et al. Survey of neonatal unit outbreaks in North London: identifying causes and risk factors. *J Hosp Infect.* (2014) 88:149–55. doi: 10.1016/j.jhin.2014.06.012
9. Dramowski A, Aucamp M, Bekker A, Mehtar S. Infectious disease exposures and outbreaks at a South African neonatal unit with review of neonatal outbreak epidemiology in Africa. *Int J Infect Dis.* (2017) 57:79–85. doi: 10.1016/j.ijid.2017.01.026
10. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* (2012) 18:268–81. doi: 10.1111/j.1469–0691.2011.03570.x
11. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. *J Clin Microbiol.* (2000) 38:1008–15.
12. McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *J Clin Microbiol.* (2006) 44:1141–4. doi: 10.1128/JCM.44.3.1141–1144.2006
13. Jarraud S. Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (Alleles), and human disease. *Infect. Immunity* (2002) 70:631–41. doi: 10.1128/iai.70.2.631–641.2002
14. Neyra RC, Frisancho JA, Rinsky JL, Resnick C, Carroll KC, Rule AM, et al. Multidrug-resistant and methicillin-resistant Staphylococcus aureus (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA). *Environ Health Perspect.* (2014) 122:471–7. doi: 10.1289/ehp.1306741
15. Andrews JI, Fleener DK, Messer SA, Kroeger JS, Diekema DJ. Screening for Staphylococcus aureus carriage in pregnancy: usefulness of novel sampling and culture strategies. *Am J Obstet Gynecol.* (2009) 201:396 e1–5. doi: 10.1016/j.ajog.2009.06.062
16. Bourgeois-Nicolaos N, Lucet JC, Daubie C, Benchaba F, Rajguru M, Ruimy R, et al. Maternal vaginal colonisation by Staphylococcus aureus and newborn acquisition at delivery. *Paediatr Perinat Epidemiol.* (2010) 24:488–91. doi: 10.1111/j.1365–3016.2010.01139.x
17. Top KA, Buet A, Whittier S, Ratner AJ, Saiman L. Predictors of Staphylococcus aureus rectovaginal colonization in pregnant women and risk for maternal and neonatal infections. *J Pediatric Infect Dis Soc.* (2012) 1:7–15. doi: 10.1093/jpids/pis001
18. Pinter DM, Mandel J, Hulten KG, Minkoff H, Tosi MF. Maternal-infant perinatal transmission of methicillin-resistant and methicillin-sensitive Staphylococcus aureus. *Am J Perinatol.* (2009) 26:145–51. doi: 10.1055/s-0028–1095179
19. Schaumburg F, Alabi AS, Mombo-Ngoma G, Kaba H, Zoleko RM, Diop DA, et al. Transmission of Staphylococcus aureus between mothers and infants in an African setting. *Clin Microbiol Infect.* (2014) 20:O390–6. doi: 10.1111/1469–0691.12417
20. Chatzakis E, Scoulica E, Papageorgiou N, Maraki S, Samonis G, Galanakis E. Infant colonization by Staphylococcus aureus: role of maternal carriage. *Eur J Clin Microbiol Infect Dis.* (2011) 30:1111–7. doi: 10.1007/s10096–011-1199–9
21. Lebon A, Moll HA, Tavakol M, van Wamel WJ, Jaddoe VW, Hofman A, et al. Correlation of bacterial colonization status between mother and child: the generation R study. *J Clin Microbiol.* (2010) 48:960–2. doi: 10.1128/JCM.01799–09
22. Yan X, Song Y, Yu X, Tao X, Yan J, Luo F, et al. Factors associated with Staphylococcus aureus nasal carriage among healthy people in Northern China. *Clin Microbiol Infect.* (2015) 21:157–62. doi: 10.1016/j.cmi.2014.08.023
23. Laub K, Tóthpál A, Kovács E, Sahin-Tóth J, Horváth A, Kardos S, et al. High prevalence of Staphylococcus aureus nasal carriage among children in Szolnok, Hungary. *Acta Microbiol Immunol Hung.* 65:59–72. doi: 10.1556/030.65.2018.001
24. Leshem E, Maayan-Metzger A, Rahav G, Dolitzki M, Kuint J, Roytman Y, et al. Transmission of Staphylococcus aureus from mothers to newborns. *Pediatr Infect Dis J.* (2012) 31:360–3. doi: 10.1097/INF.0b013e31824 4020e
25. Jimenez-Truque N, Tedeschi S, Saye EJ, McKenna BD, Langdon W, Wright JP, et al. Relationship between maternal and neonatal Staphylococcus aureus colonization. *Pediatrics* (2012) 129:e1252–9. doi: 10.1542/peds.2011–2308
26. Roca A, Bojang A, Camara B, Oluwalana C, Lette K, West P, et al. Maternal colonization with Staphylococcus aureus and Group B streptococcus is associated with colonization in newborns. *Clin Microbiol Infect.* (2017) 23:974–9. doi: 10.1016/j.cmi.2017.04.020

27. Lamaro-Cardoso J, de Lencastre H, Kipnis A, Pimenta FC, Oliveira LS, Oliveira RM, et al. Molecular epidemiology and risk factors for nasal carriage of staphylococcus aureus and methicillin-resistant S. aureus in infants attending day care centers in Brazil. *J Clin Microbiol.* (2009) 47:3991–7. doi: 10.1128/JCM.01322–09
28. Richard GW, Michael SN, Marin HK, Andrew FS, Mark JK, Alice B, et al. Linezolid in methicillin-resistant Staphylococcus aureus nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis.* (2012) 54: 621–9. doi: 10.1093/cid/cir895

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

frontiers in Pediatrics

MINI REVIEW
published: 11 December 2018
doi: 10.3389/fped.2018.00374

The Epidemiology and Pathogenesis of Kawasaki Disease

Anne H. Rowley and Stanford T. Shulman**

Department of Pediatrics, Northwestern University Feinberg School of Medicine, The Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, United States

Epidemiologic and clinical features of Kawasaki Disease (KD) strongly support an infectious etiology. KD is worldwide, most prominently in Japan, Korea, and Taiwan, reflecting increased genetic susceptibility among Asian populations. In Hawaii, KD rates are 20-fold higher in Japanese ethnics than in Caucasians, intermediate in other ethnicities. The age distribution of KD, highest in children <2 yo, lower in those <6 months, is compatible with infection by a ubiquitous agent resulting in increasing immunity with age and with transplacental immunity, as with some classic viruses. The primarily winter-spring KD seasonality and well-documented Japanese epidemics with wave-like spread also support an infectious trigger. We hypothesize KD pathogenesis involves an RNA virus that usually causes asymptomatic infection but KD in a subset of genetically predisposed children. CD8 T cells, oligoclonal IgA, and upregulation of cytotoxic T cell and interferon pathway genes in the coronaries in fatal KD also support a viral etiology. Cytoplasmic inclusion bodies in ciliated bronchial epithelium identified by monoclonal antibodies made from oligoclonal IgA heavy chains also supports a viral etiology. Recent availability of "second generation" antibodies from KD peripheral blood plasmablasts may identify a specific viral antigen. Thus, we propose an unidentified ("new") RNA virus infects bronchial epithelium usually causing asymptomatic infection but KD in a subset of genetically predisposed children. The agent persists in inclusion bodies, with intermittent respiratory shedding, entering the bloodstream via macrophages targeting coronaries. Antigen-specific IgA plasma cells and CD8 T cells respond but coronaries can be damaged. IVIG may include antibody against the agent. Post infection, 97–99% of KD patients are immune to the agent, protected against recurrence. The agent can spread either from those with asymptomatic primary infection in winter-spring or from a previously infected contact who intermittently sheds the agent.

Keywords: kawasaki, pathogenesis, etiology, pediatric, coronary

OPEN ACCESS

Edited by:
Miika Kaleva Arvonen, Kuopio University Hospital, Finland

Reviewed by:
Audrey Dionne, Harvard Medical School, United States
Nagib Dahdah, CHU Sainte-Justine, Canada
Toru Watanabe, Niigata City General Hospital, Japan
Dirk Van Gysel, Onze Lieve Vrouwziekenhuis Hospital, Belgium

Correspondence:
Anne H. Rowley
arowley@luriechildrens.org
Stanford T. Shulman
sshulman@northwestern.edu

Specialty section:
This article was submitted to Pediatric Infectious Diseases, a section of the journal Frontiers in Pediatrics

Received: *12 October 2018*
Accepted: *15 November 2018*
Published: *11 December 2018*

Citation:
Rowley AH and Shulman ST (2018) The Epidemiology and Pathogenesis of Kawasaki Disease. Front. Pediatr. 6:374. doi: 10.3389/fped.2018.00374

EPIDEMIOLOGY

Both epidemiologic and clinical features of Kawasaki Disease (KD) strongly support an Infectious etiology. The clinical features of KD including fever, rash, mucosal changes, conjunctival erythema, and cervical lymphadenopathy are all compatible with an infectious illness, and many common (predominantly viral) infections by necessity are included in the differential diagnosis of KD.

Kawasaki Disease (KD) is a worldwide illness, with varying incidence rates that primarily reflect the racial composition of the populations of various countries. The highest incidence of KD is in

Japan, and this has steadily increased with an annual rate of 308.0 per 100,000 children under 5 years reported in 2014 (1). In Japan one in 65 children develops KD by age 5 years. The second highest reported rate was 199.7 per 100,000 <5 years old in 2014 in South Korea (2), while Taiwan has the third highest rate, 82.8 per 100,000 <5 years old in 2010 (3). In countries with predominantly non-Asian populations, the usual annual rate is 10–20 per 100,000 <5 years old (4).

More than 15,979 cases of KD were reported in Japan in 2015, with local clusters occurring commonly, unlike the nationwide epidemics that occurred in 1979, 1982, and 1985–86 (1, 5). In those epidemics, there appeared to be wave-like spread from one prefecture to an adjacent one, a pattern very similar to the spread of specific viral illnesses like measles, for example, in Japan, thus strongly supporting an infectious etiology of KD. In Hawaii, with its complex multi-racial and multi-ethnic population, the overall annual KD incidence is about 50.4/100,000 <5 y/o; for Japanese ethnic children in Hawaii, the rate is about 210.5 and for Caucasians about 13.7, with intermediate rates for children of native Hawaiian, Chinese, Filipino and other Asian ancestries (6). The very striking differences in ethnic-specific rates are indicative of a very strong genetic basis of susceptibility.

The ratio of male: female KD patients approximates 1.5:1 in virtually all countries (1, 4), and severe cardiac complications of KD are even more significantly overrepresented in males. The basis of the male preponderance is unclear but similar to that observed in many infectious diseases.

Kawasaki Disease (KD) has a striking age distribution, with almost 100% of cases occurring in children, 80% in children <5 years old, and 50% in those <2 years old. In a recent Japanese survey 0.7% of cases were ≥ 10 years old (1). The age-incidence curve of KD may help to elucidate risk factors and appears compatible with a ubiquitous highly transmissible infectious agent, and is similar to that seen with respiratory syncytial virus (RSV), for example. The peak age of KD is approximately 10–11 months of life, with a relatively low incidence in the first 6 months, suggesting both the possibility of transplacental immunity as seen in many classic infectious illnesses, as well as progressively increasing degrees of immunity to the KD agent throughout childhood.

The seasonality of KD, with winter peaks in Japan and winter-spring predominance in the US and many other temperate areas, is highly suggestive of a viral (probably respiratory viral) etiology (4, 7). Some reports have suggested summer and winter peaks (Beijing and Shanghai), or spring peaks (Sichuan and Hong Kong), while no clear seasonality has been seen in Hawaii (6), and winter predominance was reported from at least some Southern Hemisphere countries. Despite the observed seasonality, in most areas sporadic cases are recognized throughout the year, contrasting somewhat with the usual patterns commonly seen with many highly transmissible respiratory viral illnesses. Recurrent KD is defined as a new illness that meets KD criteria beginning at least 3-months and usually within 2 years after an initial episode of KD, when levels of inflammatory markers have completely normalized. Recurrence occurs in about 1% or fewer of all KD patients, and in up to 3% of those of Asian ethnicity (8).

During an outbreak of KD on Mikayo Island, Japan, in 1980–1981 (a fairly isolated population of ∼80,000 at that time), 9 KD cases were diagnosed in a 1 month period, and 4 of the cases had close geographic and social contacts, supporting the possibility of direct person-to-person transmission of a KD etiologic agent (9). While there is limited other direct evidence to indicate that KD can be transmitted from person to person, for example in a daycare setting, much circumstantial evidence supports an infectious etiology with genetically susceptible individuals manifesting the clinical features of KD and others having trivial or no symptoms. Simultaneous or sequential cases in siblings, twins, or other contacts are reported, especially during Japanese outbreaks (10). In Japan, secondary sibling cases occur at rates substantially higher than the general childhood population. Sibling cases are reported more frequently in twins than in non-twins, suggesting both genetic susceptibility and person to person transmission. Japanese family data suggest that sibling cases tend to cluster either on the same day as the index case or 7 days later (11).

History of increased frequency of antecedent respiratory illnesses in KD compared to controls was documented in the 1980's in several outbreak investigations (12, 13). Together with the epidemiologic features noted above, the clinical features characteristic of KD also strongly suggest that an infectious agent, perhaps one that has not yet been identified as a human pathogen, is etiologically related to KD.

PATHOGENESIS

The epidemiologic features of KD described above strongly support infection with a ubiquitous agent that usually results in asymptomatic infection, but causes KD in a small subset of genetically predisposed children. The occurrence of epidemics and geographic wave-like spread of KD during epidemics supports a presently unknown single agent or closely related group of agents as the etiology. The failure of KD patients to respond to antibiotic therapy makes a viral etiology more likely than a bacterial cause. Moreover, the prevalence of CD8 T cells in the inflammatory infiltrate and the upregulation of cytotoxic T cell and interferon pathway genes in the coronary arteries of children who have died of KD are very suggestive of a viral etiology (14, 15).

We discovered an oligoclonal IgA response in the coronary arteries of children who died from KD, and we made "first generation" KD synthetic antibodies using oligoclonal IgA heavy chains with random light chains (16–19). These "first generation" antibodies detected antigen residing in ciliated bronchial epithelium in KD lung and in a subset of macrophages in KD but not in infant control tissues by immunohistochemistry; the antigen in lung localized to intracytoplasmic inclusion bodies that were identified using stains for protein and for RNA (20–22). The inclusion bodies were identified in children from the US and Japan using a single monoclonal antibody, strongly suggesting a single infectious agent as the cause (20, 22, 23). The inclusion bodies could also be identified in some KD children who died as late as months to years after onset (21). Further investigations of

acute phase KD lung samples showed upregulation of interferon pathway genes and virus-like particles in close proximity to the inclusion bodies by transmission electron microscopy (23). However, these antibodies did not identify the specific antigen by Western blot and immunoprecipitation assays, likely because of a lack of cognate heavy and light chain partners in these "first generation" antibodies. This problem has been recently overcome by preparing "second generation" antibodies from acute KD peripheral blood plasmablasts, which include cognate light and heavy chain partners and show great promise in identifying specific antigen (24).

One theory presently favored by some is that KD can result from infection with any of a wide range of infectious agents in a genetically predisposed host, and some investigators propose an immune defect in KD children. We believe that these theories fail to explain epidemiologic findings in KD. If multiple agents can cause KD, epidemics would either not be observed or specific known infectious agents would be identified by careful epidemiologic study as being associated with the epidemics. In fact, there has been an absence of association of KD with known infectious agents during epidemics and outbreaks despite careful study by epidemiologists in Japan, in the US at the Centers for Disease Control, and in other nations (5, 12, 13, 25). If any of multiple agents can cause KD, a substantially higher recurrence rate than the observed 1–3% in the US and Japan would be likely. Because the vast majority of patients do not develop other health problems following KD, an immune defect seems highly unlikely.

Our studies demonstrating an antigen-driven IgA immune response in acute KD and the presence of KD antigen in intracytoplasmic inclusion bodies in KD bronchial epithelium lead us to put forth the following model of KD pathogenesis (**Figure 1**). We propose that a presently unidentified (likely "new") RNA virus infects ciliated bronchial epithelium, causing asymptomatic infection in most individuals and KD in a small subset of genetically predisposed children. Children <6 months of age are less susceptible because of passive maternal antibody. The virus can result in sporadic cases of KD or in outbreaks. The agent can remain persistent in cytoplasmic inclusion bodies, with intermittent shedding into the respiratory tract of previously infected individuals. It can enter the bloodstream via macrophages and target particularly the coronary arteries and also other sites. Antigen-specific IgA plasma cells (17, 19, 20, 22, 23) and CD8 T cells (14, 15, 26) respond to the infection, but coronary arteries can be damaged. The provision of specific antibodies directed at the ubiquitous KD agent could explain the efficacy of intravenous gammaglobulin (IVIG) in the treatment of KD. These specific antibodies are present in IVIG because most adult donors were asymptomatically infected during young childhood, which accounts for the reduced prevalence in older children and the rarity of KD in adults. After infection, 97–99% of KD patients are immune to the agent and do not have a recurrence of KD. The agent can be spread through the population either from community contacts with asymptomatic primary infection particularly in the winter-spring, or from a close contact who had been previously infected and then intermittently sheds the agent, resulting in cases during other seasons. We believe that our model, although speculative, fits clinical and epidemiologic findings in KD much better than other currently proposed speculative models.

Identification of the etiology of KD is the most important research goal in the field. With this information, a diagnostic

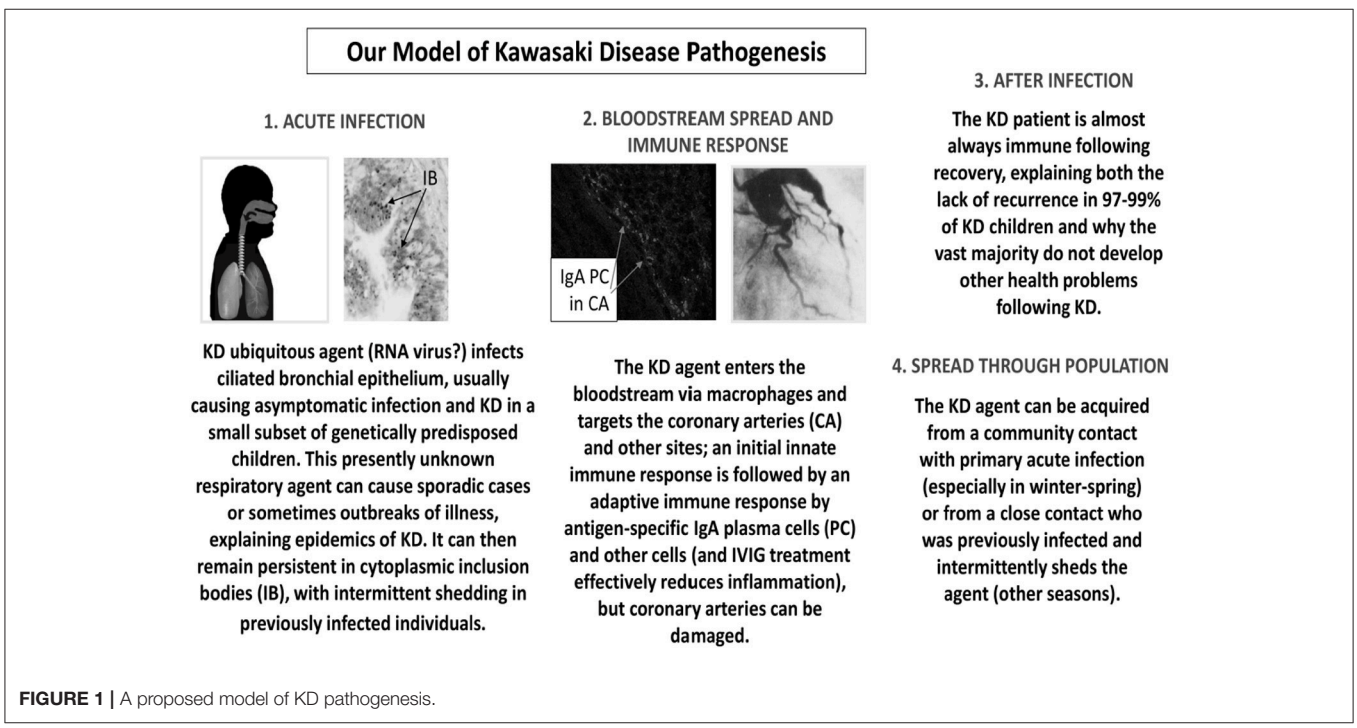

FIGURE 1 | A proposed model of KD pathogenesis.

test can be developed, therapy improved, and prevention become possible. Hopefully, in the near future, the etiology can be discovered using synthetic antibodies derived from KD patients' B cell immune response to the triggering agent.

AUTHOR CONTRIBUTIONS

AR and SS contributed equally to conceiving the topics covered and in authoring the work.

REFERENCES

1. Makino N, Nakamura Y, Yashiro M, Sano T, Ae R, Kosami K, et al. Epidemiological observations of Kawasaki disease in Japan, 2013-2014. *Pediatr Int*. (2018) 60:581–7. doi: 10.1111/ped.13544
2. Kim GB, Han JW, Park YW, Song MS, Hong YM, Cha SH, et al. Epidemiologic features of Kawasaki disease in South Korea: data from nationwide survey, 2009-2011. *Pediatr Infect Dis J*. (2014) 33:24–7. doi: 10.1097/INF.0000000000000010
3. Lin MC, Lai MS, Jan SL, Fu YC. Epidemiologic features of Kawasaki disease in acute stages in Taiwan, 1997-2010: effect of different case definitions in claims data analysis. *J Chin Med Assoc*. (2015) 78:121–6. doi: 10.1016/j.jcma.2014.03.009
4. Holman RC, Belay ED, Christensen KY, Folkema AM, Steiner CA, Schonberger LB. Hospitalizations for Kawasaki syndrome among children in the United States, 1997-2007. *Pediatr Infect Dis J*. (2010) 29:483–8. doi: 10.1097/INF.0b013e3181cf8705
5. Yanagawa H, Nakamura Y, Kawasaki T, Shigematsu I. Nationwide epidemic of Kawasaki disease in Japan during winter of 1985-86. *Lancet* (1986) 2:1138–9. doi: 10.1016/S0140-6736(86)90541-6
6. Holman RC, Christensen KY, Belay ED, Steiner CA, Effler PV, Miyamura J, et al. Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. *Hawaii Med J*. (2010) 69:194–7.
7. Yanagawa H, Nakamura Y, Yashiro M, Fujita Y, Nagai M, Kawasaki T, et al. A nationwide incidence survey of Kawasaki disease in 1985-1986 in Japan. *J Infect Dis*. (1988) 158:1296–301. doi: 10.1093/infdis/158.6.1296
8. Nakamura Y, Hirose K, Yanagawa H, Kato H, Kawasaki T. Incidence rate of recurrent Kawasaki disease in Japan. *Acta Paediatr*. (1994) 83:1061–4. doi: 10.1111/j.1651-2227.1994.tb12986.x
9. Takeuchi S, Yanagawa H, Kawasaki T, Yanase Y. An outbreak of Kawasaki disease in Miyako Island in Okinawa prefecture. *Pediatr Int*. (1983) 25:436–7. doi: 10.1111/j.1442-200X.1983.tb01741.x
10. Kottek A, Shimizu C, Burns JC. Kawasaki disease in monozygotic twins. *Pediatr Infect Dis J*. (2011) 30:1114–6. doi: 10.1097/INF.0b013e31822ac4ff
11. Fujita Y, Nakamura Y, Sakata K, Hara N, Kobayashi M, Nagai M, et al. Kawasaki disease in families. *Pediatrics* (1989) 84:666–9.
12. Bell DM, Brink EW, Nitzkin JL, Hall CB, Wulff H, Berkowitz ID, et al. Kawasaki syndrome: description of two outbreaks in the United States. *N Engl J Med*. (1981) 304:1568–75. doi: 10.1056/NEJM198106253042603
13. Dean AG, Melish ME, Hicks R, Palumbo NE. An epidemic of Kawasaki syndrome in Hawaii. *J Pediatr*. (1982) 100:552–7. doi: 10.1016/S0022-3476(82)80751-8
14. Brown TJ, Crawford SE, Cornwall ML, Garcia F, Shulman ST, Rowley AH. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *J Infect Dis*. (2001) 184:940–3. doi: 10.1086/323155
15. Rowley AH, Wylie KM, Kim KY, Pink AJ, Yang A, Reindel R, et al. The transcriptional profile of coronary arteritis in Kawasaki disease. *BMC Genomics* (2015) 16:1076. doi: 10.1186/s12864-015-2323-5
16. Rowley AH, Eckerley CA, Jack HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. *J Immunol*. (1997) 159:5946–55.
17. Rowley AH, Shulman ST, Garcia FL, Guzman-Cottrill JA, Miura M, Lee HL, et al. Cloning the arterial IgA antibody response during acute Kawasaki disease. *J Immunol*. (2005) 175:8386–91. doi: 10.4049/jimmunol.175.12.8386
18. Rowley AH, Shulman ST, Mask CA, Finn LS, Terai M, Baker SC, et al. IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. *J Infect Dis*. (2000) 182:1183–91. doi: 10.1086/315832
19. Rowley AH, Shulman ST, Spike BT, Mask CA, Baker SC. Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. *J Immunol*. (2001) 166:1334–43. doi: 10.4049/jimmunol.166.2.1334
20. Rowley AH, Baker SC, Shulman ST, Fox LM, Takahashi K, Garcia FL, et al. Cytoplasmic inclusion bodies are detected by synthetic antibody in ciliated bronchial epithelium during acute Kawasaki disease. *J Infect Dis*. (2005) 192:1757–66. doi: 10.1086/497171
21. Rowley AH, Baker SC, Shulman ST, Garcia FL, Fox LM, Kos IM, et al. RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after acute Kawasaki disease. *PLoS ONE* (2008) 3:e1582. doi: 10.1371/journal.pone.0001582
22. Rowley AH, Baker SC, Shulman ST, Garcia FL, Guzman-Cottrill JA, Chou P, et al. Detection of antigen in bronchial epithelium and macrophages in acute Kawasaki disease by use of synthetic antibody. *J Infect Dis*. (2004) 190:856–65. doi: 10.1086/422648
23. Rowley AH, Baker SC, Shulman ST, Rand KH, Tretiakova MS, Perlman EJ, et al. Ultrastructural, immunofluorescence, and RNA evidence support the hypothesis of a "new" virus associated with Kawasaki disease. *J Infect Dis*. (2011) 203:1021–30. doi: 10.1093/infdis/jiq136
24. Ho IY, Bunker JJ, Erickson SA, Neu KE, Huang M, Cortese M, et al. Refined protocol for generating monoclonal antibodies from single human and immune B cells. *J Immunol Meth*. (2016) 438:67–70. doi: 10.1016/j.jim.2016.09.001
25. Salo E, Pelkonen P, Pettay O. Outbreak of Kawasaki syndrome in Finland. *Acta Paediatr Scand*. (1986) 75:75–80. doi: 10.1111/j.1651-2227.1986.tb10160.x
26. Choi IH, Chwae YJ, Shim WS, Kim DS, Kwon DH, Kim JD, et al. Clonal expansion of CD8+ T cells in Kawasaki disease. *J Immunol*. (1997) 159:481–6.

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

frontiers in Pediatrics

REVIEW
published: 05 February 2019
doi: 10.3389/fped.2019.00018

Causes of Kawasaki Disease—From Past to Present

*Satoru Nagata**

Departments of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan

Kawasaki disease (KD) is a multisystem vasculitis that primarily affects the coronary arteries of young children. The causes of KD remain a mystery. It is suspected that some sort of infectious agent is involved because KD has epidemicity and seasonality. That said, the incidence of the disease is high among Japanese people, so it can be speculated that the hosts may have some sort of genetic characteristic that leaves them susceptible to KD. Various theories regarding the etiology have been asserted, such as the infectious vasculitis theory, autoantigen theory, superantigen theory, and RNA virus theory; however, none of them have been able to overcome this epidemicity. Taking into consideration the knowledge gained from previous reports, the best scenario explaining the pathogenesis is "individuals with certain genetic backgrounds are affected by microorganisms which trigger KD." In this article, the pathogenesis of KD is discussed with a focus on the microorganisms mentioned above, along with the previous and current hypotheses as well as my own opinion.

Keywords: Kawasaki disease, etiology, superantigens, heat-shock proteins, epidemicity

OPEN ACCESS

Edited by:
Hiromichi Hamada,
Tokyo Women's Medical University Yachiyo Medical Center, Japan

Reviewed by:
Antonio Condino-Neto,
University of São Paulo, Brazil
Hermann Girschick,
Vivantes Klinikum, Germany

***Correspondence:**
Satoru Nagata
nagata.satoru@twmu.ac.jp

Specialty section:
This article was submitted to Pediatric Immunology, a section of the journal Frontiers in Pediatrics

Received: *31 October 2018*
Accepted: *16 January 2019*
Published: *05 February 2019*

Citation:
Nagata S (2019) Causes of Kawasaki Disease—From Past to Present. Front. Pediatr. 7:18. doi: 10.3389/fped.2019.00018

INTRODUCTION

The etiology of Kawasaki disease (KD) has remained a mystery since Dr. Tomisaku Kawasaki proposed the disease in 1967. A number of epidemiological and clinical observations suggest that KD is caused by an infectious agent, with suggestions ranging from Staphylococci, Streptococci, Mycoplasma, or Chlamydia (1–4), to viruses such as adenovirus, parvovirus, or Epstein-Barr virus (5–7). It is suspected that infection is involved because KD has epidemicity and seasonality. That said, the incidence of the disease is high among Japanese people, so it can be speculated that hosts may have some sort of genetic characteristic that leaves them susceptible to KD. Taking into consideration the knowledge gained from previous reports, the best scenario explaining the pathogenesis is "individuals with certain genetic backgrounds are affected by certain microorganisms which trigger KD." This article provides an overview along with the newly acquired knowledge by Nagata et al. (8), with a focus on +pathogenic organisms.

Possible Pathogens

Various pathogens have been proposed as the trigger, but none have been decisively established. One of the reasons is that there are a variety of items that must be explained to determine that the etiology is certain [**Table 1**, (8)]. In particular, the epidemiology, wherein "the incidence is high among Japanese people," shall be most difficult to explain.

TABLE 1 | Epidemiological conditions that the pathogen of Kawasaki disease must meet (8).

1. EPIDEMIOLOGICAL CONDITIONS
Frequently observed in infants aged four or younger
Moderate epidemicity
Frequently observed in Japanese people
2. PATHOLOGICAL CONDITIONS
Includes six types of characteristic clinical symptoms
Redness in the BCG region
Cures within 2–3 weeks (self-limited)
Coronary artery lesions occur
Exhibits hematologically strong inflammation findings in the acute stage
Blood platelets increase in the convalescent stage
Antimicrobial agents are ineffective

Such pathogens must overcome the following challenges, in order, from low to high level of difficulty: (1) detected at a high frequency in a patient; (2) symptoms can be explained; (3) coronary artery lesions (CALs) can be explained; and (4) epidemiological conditions can be explained.

Various Theories Regarding the Etiology

A common point of view among infectious theories at an early stage is that a pathogen is recognized as an antigen-presenting cell (APC), with factors such as tumor necrosis factor (TNF) α, interleukin (IL)-6, vascular endothelial growth factor (VEGF) (9) produced by macrophage and T cells, etc. activated by macrophages causing vasculitis, leading to the formation of the pathology (**Figure 1A**). Although there are many pediatric diseases mainly involving vasculitis, few diseases besides KD cause CALs. Therefore, many researchers assume the existence of an autoantigen that becomes the target of these attack factors in the components of the vessel wall of small and medium-sized arteries such as the coronary artery (**Figure 1B**). However, the autoantigen involved remains uncertain.

Superantigen Theory

According to previous reports on Staphylococcus, hemolytic streptococcus, and Yersinia, the generation of superantigens is involved in the pathology of KD in each report (10–15) (**Figure 1C**). The primary symptoms of KD such as fever, oral cavity findings, and exanthema as well as aggravation of the serum inflammatory reaction are similar to those of diseases related to superantigens. However, the formation of CALs, as the most typical characteristic of KD, cannot be observed in diseases related to superantigens (16).

RNA Virus Theory

Rowley et al. have reported that the postmortem examination of KD patients revealed the invasion of many mononucleoses, CD8 positive T-cells, and IgA producing plasma cells (17), proposing a hypothesis that viruses invading mainly from the respiratory apparatus stimulate CD8 positive T-cells and B cells in the organ lymph nodes and differentiate them into IgA producing plasma cells, with these cell groups giving rise to vasculitis (**Figure 1D**). Recent studies have shown that respiratory viruses are detected by a PCR in up to half of KD patients (18, 19) and an ultrastructural search suggested the possibility of a respiratory virus in autopsy specimens (20, 21); however, no virus has ever been repeatedly confirmed in such studies (22).

Superantigen + Heat-Shock Protein Theory

The authors have hypothesized the gastrointestinal (GI) tract microbiota could be involved in KD because of the following reasons: (1). GI tract is constantly exposed to a milieu of microorganisms, various antigens, and other agents, (2). It is the largest lymphoid tissue in the body, and (3). It has not been fully investigated due to some technical problems. Therefore, we focused on the mucous membrane of the GI tract with a vast area invaded by antigens and enhanced mucosal immunity, and conducted a biopsy on the mucous membrane of the duodenum of infants with KD, suggesting that certain types of antigens may have invaded that significantly activate the immunologic system of the host (23). We suspected that the possible antigens causing the disease may be a virulent alpha hemolytic streptococcus and Staphylococcus, which produce superantigens using the T-cell receptors of Vβ2 repertoire of the GI tract mucosa of infant patients 1 (24, 25). However, the problem we faced is that superantigens cannot explain the formation of coronary artery lesions and differences in race among patients with KD. In 2005, the authors conducted the following analysis (8), hinting at the theory that "superantigens cause the explosive activation of T-cells, producing autoreactive cytotoxic T-cells, which attack autoantibodies expressed on vascular endothelial cells, resulting in vasculitis" (16).

Knowledge Obtained From the Analysis by the Authors

First, culture supernatants of bacteria isolated from the oral cavity or duodenal mucosa of 19 children with KD were added to peripheral blood mononuclear cells of the same host, from which we selected those promoting significant cell proliferation using ^{3}H-thymidine uptake (21–23). As a result, six kinds (18 strains) of gram-positive cocci and five kinds (13 strains) of gram-negative bacteria which remarkably proliferate the peripheral blood mononuclear cells of the same patient child were isolated from the oral mucosa/duodenal mucosa of the patient. In 12 of 19 patients, both gram-positive cocci and gram-negative bacteria were isolated, including patients complicated with CALs. These bacteria showed remarkable tolerance to antibiotics. An examination of the superantigen activity by flow cytometry revealed that the isolated gram-positive cocci induced a marked increase in T cells using the Vβ2 repertoire of the host peripheral blood (26, 27). Furthermore, we detected the reactive protein between the co-culture supernatant with mononuclear cells and the host serum using the Western blotting method (28) (**Figure 2**). In the gram-negative bacteria, while a large amount of bacteria-specific heat-shock protein (HSP) 60 was generated in the acute phase of KD, the production of a large amount of human HSP60 was induced in the peripheral blood of the

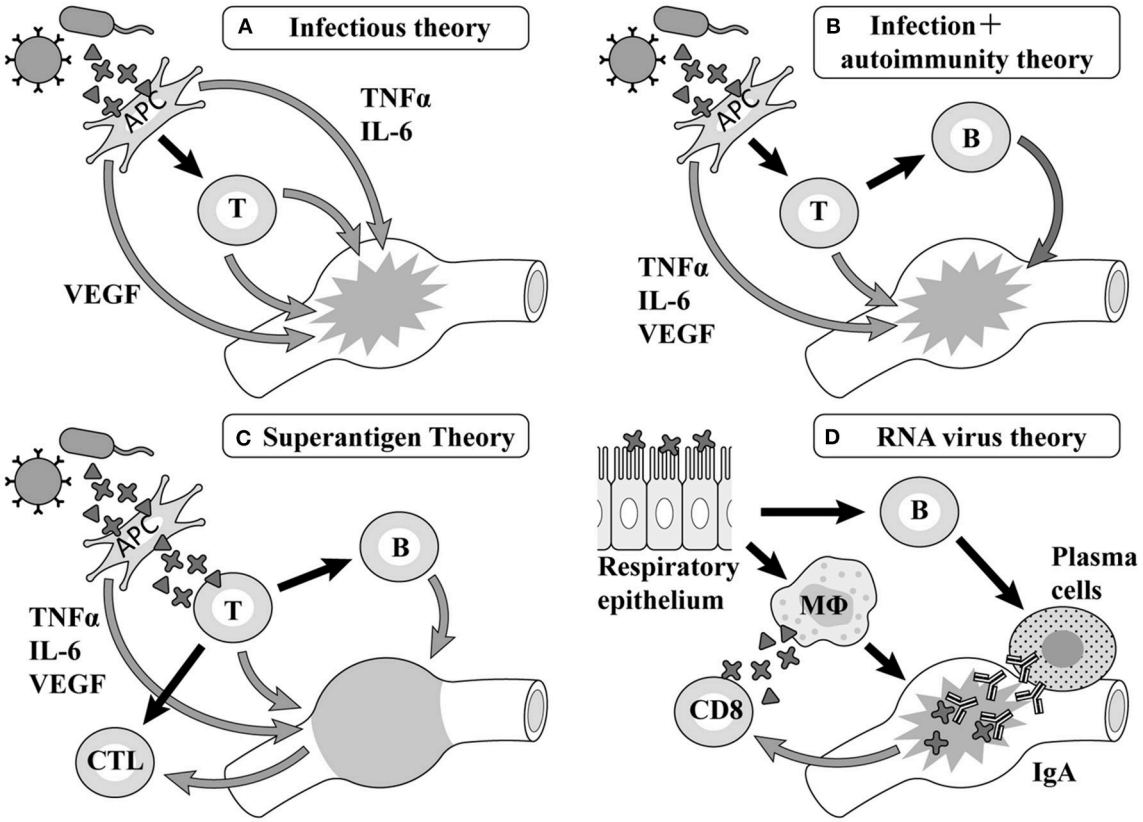

FIGURE 1 | Etiology hypotheses and mechanisms. TNF, tumor necrosis factor; IL, interleukin; VEGF, vascular endothelial growth factor; APC, antigen-presenting cells; MΦ, macrophages.

infant patients. In addition, both gram-positive cocci and gram-negative bacteria caused the secretion of inflammatory cytokine: interferon gamma (IFNγ) and TNFα, in the peripheral blood mononuclear cells of the hosts. On the other hand, gram-negative bacteria induced the production of anti-inflammatory cytokine, IL-10 in the peripheral blood mononuclear cells of the patients (29).

The Pathogenesis of KD Presumed From the Results of Our Analysis (Figure 3)

At first, once gram-positive cocci with superantigen activity infect the host from the mouth, they stimulate gram-negative bacteria in the upper GI tract and promote the production of HSP60. The superantigen stimulates Th1 cells causing inflammation and also induces the production of autoreactive B cells and cytotoxic T cells. With vascular endothelial cells, the stimulation of HSP60 produced by gram-negative bacteria promotes the production of human HSP 60, which plays a cytoprotective role in the nucleus and cytoplasm and is arranged so as to penetrate the membrane on the surface of vascular endothelial cells. Since part of human HSP 60 has a molecular structure derived from bacteria and the other part has molecular structure specific to human beings, human HSP 60 becomes a target of autoantibodies and cytotoxic T cells produced by autoreactive B cells generated by superantigens. The membrane of vascular endothelial cells is destroyed, causing CALs (30–34). Subsequently, human HSP60 is extracellularly released and the molecular structure derived from bacteria activates Th1

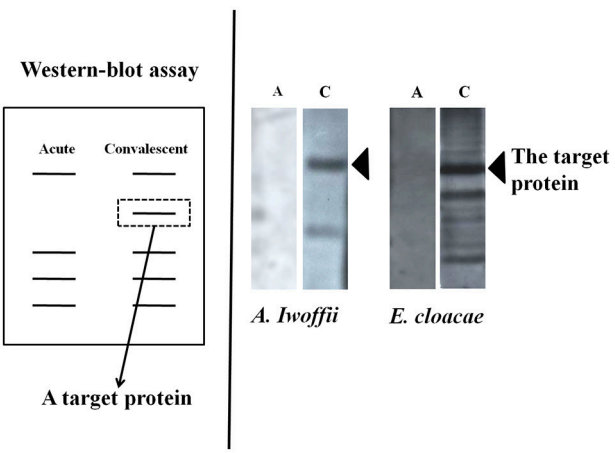

FIGURE 2 | Detection of antibodies responses to bacterial products. The target proteins could be produced in the acute phase of Kawasaki disease and neutralized by intravenous immunoglobulin in the convalescent phase.

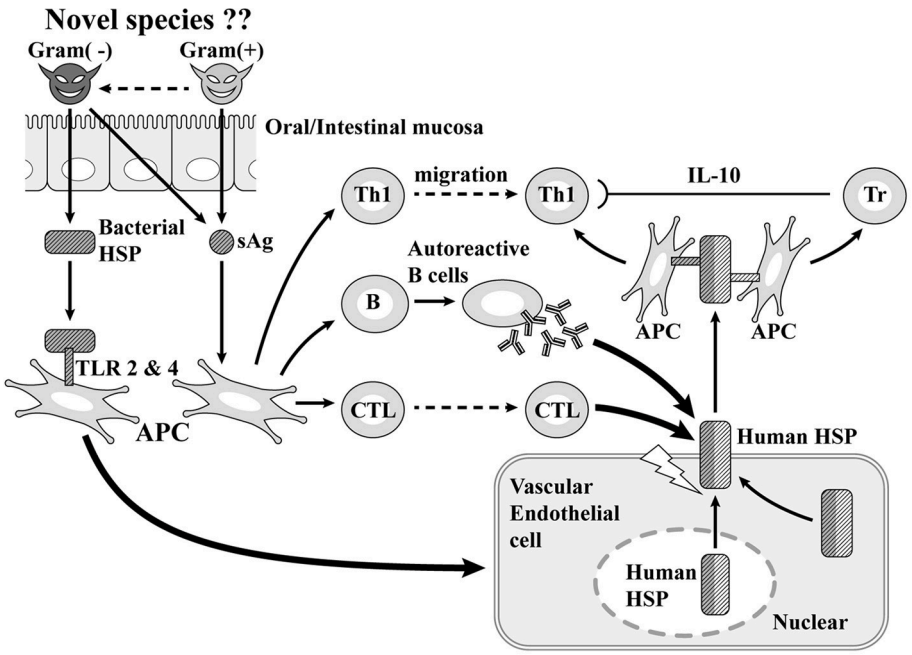

FIGURE 3 | Superantigen (sAg) + coronary artery lesion due to heat-shock protein (HSP; our theory). TLR, Toll-like receptor; Tr, regulatory T cell.

cells, while the molecular structure specific to humans activates regulatory T cells. The former further contributes to vasculitis through the secretion of IFNγ, with the latter suppressing the excessive activation of Th1 cells through the secretion of anti-inflammatory cytokine, IL-10 (35). Epidemiological and immunopathological studies have suggested that HSP60 autoantibodies can cross-react with bacterial and self-HSP 60 and induce cytotoxic damage of stressed endothelial cells, resulting in coronary atherosclerosis (31–34). These theories appear applicable to the pathogenesis of coronary lesions of KD considering the high HSP 60 expression in endothelial cells together with the serological detection of bacterial and self-HSP in patients with KD (36–38). Vascular surface-expressed HSP60 the transfer of which from the cytoplasm or mitochondria could be induced by bacterial HSP60 stimulation, can be recognized by circulating anti-HSP autoantibodies or cytotoxic T lymphocytes targeting autoantigens. The incidence of coronary lesions in patients with KD may depend upon how strongly causative agents can induce the initial immune activation that elicits autoreactive T cells and importantly, the number of self-HSP molecules they can evoke from the cytoplasm or mitochondria to the vascular surface. Gram-negative microbes appeared to trigger more self-HSP than Gram-positive cocci. Actually, Gram-negative microbes such as *N. mucosa* coexisting with Gram-positive cocci have been isolated in KD patients with vascular involvement.

Why the Incidence Is High in Japanese People

The amino acid sequences of *Neisseria* HSP60 221–231 and 255–269 had high homology (60 and 50%, respectively) with self-HSP 60 246–256 and 280–294, which are core protein epitopes with high capability to bind to human class II molecules, to induce the production of IFNγ and IL-10, respectively (29). These sequences were demonstrated to have high affinity with HLA-DRB1 *0401, the gene products of which are recognized by HLA-DR4, which is detected by DR-peptide binding assays. Therefore, the higher incidence of KD is due to the affinity between the binding site with MHC class II molecules on bacterial HSP60 and HLA-DR4 (39–41) (**Figure 4**). The frequency of having this subclass of MHC class II of HLA-DR4 varies between races and has been reported as being typically high in Japanese people (42). This may account for the high incidence of KD among Japanese. Korea and Taiwan have the second and third highest annual incidences of KD in the world, which may support this hypothesis (43, 44).

Regional differences in the risk-allele frequencies of some susceptibility single nucleotide polymorphisms (SNPs) have been identified in genes such as caspase-3 (CASP3) and inositol 1,4,5-triphosphate kinase-C(ITPKC) (45, 46); however, none of the associated parameters proved to be informative in predicting the onset of KD or the development of coronary artery complications (47, 48).

Antimicrobial Therapy to KD

The background of our study was based on the hypothesized mechanisms underlying the efficacy of intravenous immunoglobulin (IVIG), which include neutralization of the etiologic agents as well as the immunomodulation of T cell regulation and a reduction in the productions of inflammatory cytokines, such as TNFα (49). The levels of IL-6, IFNγ, and TNFα have been reported to be significantly increased before IVIG treatment. While those of IL-6, IL-10, and IFNγ rapidly

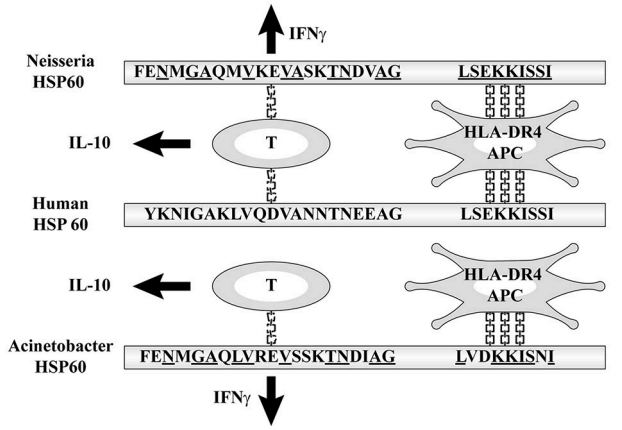

FIGURE 4 | Affinity between HLA-DR4 and the binding site with MHC class II molecules on bacterial HSP60.

decreased after treatment (50). The level of TNFα significantly reduced after treatment in KD patients without coronary artery lesions (CALs); however, it was still high in those with CALs and in patients with IVIG-resistant disease (51). Corticosteroids have been considered for such patients as a representative adjunctive therapy that has the potential to non-specifically reduce inflammatory cytokine production; however, such treatment also has the potential to induce hypercoagulopathy at the time of thrombus formation in CALs. Most the other agents used for adjunctive therapies, such as single infusion of infliximab and cyclosporine, are administered because they are effective for reducing inflammatory cytokine production; however, these are nothing more than symptomatic treatments.

In our study, we found particular Gram-negative microbes producing HSP 60 and several Gram-positive cocci possessing superantigenic properties on the surface of the GI tract that might be involved in the onset of KD. We showed that these organisms were all resistant to commonly used antibiotics, except for sulfamethoxazole trimethoprim (SMX-TMP). We used SMX-TMP for seven cases of KD that were unresponsive to IVIG and studied the antipyretic potency of this treatment. In six out of the seven cases, antipyretic potency was observed without side effects within 2 days of the initial administration (52). Antimicrobial therapy using SMX-TMP may therefore represent a novel strategy for treating cases of KD that are unresponsive to IVIG.

Future Prospects

The key to solving the mystery of the pathology of KD is likely to be hidden in CALs as the primary lesion. Aneurysmorrhaphy has interesting characteristics in that the intima and the adventitia are invaded before the media, along with the fact that the blood vessels distributing outside the organ are invaded, although the main characteristic is the destruction of the media forming the framework. We intend to detect possible pathogens on the upper intestinal mucosa or in the peripheral blood using a highly sensitive microbial analytical system based on reverse transcription-quantitative PCR (53). Going forward, we think it is desirable to launch a multicenter collaborative project involving the registration, preservation and search for autopsy specimens, etc.

CONCLUSION

Currently, among the numerous etiologies of KD, the most credible theory is that bacterial infection triggers KD. However, we should also consider the possibility of fungi and new types of viruses, which are pathogens that have not yet been paid attention to Rowley (54). To this end, the application of new technologies is expected, such as comprehensive analyses and microarrays. In addition, we would like to emphasize that therapeutic validation should be conducted for possible pathogens, including confirming the effectiveness of antimicrobial agents, etc., in order to make the etiology theory useful in practice.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This work was supported by Secom Science and Technology Foundation.

ACKNOWLEDGMENTS

I wish to express extreme gratitude to Prof. Yuichiro Yamashiro and Dr. Yoshikazu Ohtsuka, Juntendo University Graduate School of Medicine, Tokyo for offering many valuable advices.

REFERENCES

1. Matsubara K, Fukaya T, Miwa K, Shibayama N, Nigami H, Harigaya H, et al. Development of serum IgM antibodies against superantigens of *Staphylococcus aureus* and *Stretococcus pyogenes* in Kawasaki disease. *Clin Exp Immunol*. (2006) 143:427–34. doi: 10.1111/j.1365-2249.2006.03015.x
2. Barton M, Melbourne R, Morais P, Christie C. Kawasaki syndrome associated with group A streptococcal and Epstein–Barr virus co-infections. *Ann Trop Paediatr*. (2002) 22:257–60. doi: 10.1179/027249302125001543
3. Wang JN, Wang SM, Liu CC, Wu JM. *Mycoplasma pneumoniae* infection associated with Kawasaki disease. *Acta Paediatr*. (2001) 90:594–5. doi: 10.1111/j.1651-2227.2001.tb00810.x
4. Normann F, Nääs J, Gnarpe J, Bäckman H, Gnarpe H. Demonstration of *Chlamydia pneumoniae* in cardiovascular tissue from children with Kawasaki disease. *Pediatr Infect Dis J*. (1999) 18:72–3.
5. Embil JA, McFarlane ES, Murphy DM, Krause VW, Stewart HB. Adenovirus type 2 isolated from a patient with fatal Kawasaki disease. *Can Med Assoc J*. (1985) 132:1400.
6. Holm JM, Hansen LK, Oxhøj H. Kawasaki disease associated with parvovirus B19 infection. *Eur J Pediatr*. (1995) 154:633–4. doi: 10.1007/BF02079066
7. Kanegane H, Tsuji T, Seki H, Yachie A, Yokoi T, Miyawaki T, et al. Kawasaki disease with a concomitant primary Epstein–Barr virus infection. *Acta Paediatr Jpn*. (1994) 36:713–6. doi: 10.1111/j.1442-200X.1994.tb03277.x

8. Nagata S, Yamashiro Y, Ohtsuka Y, Shimizu T, Sakurai Y, Misawa S, et al. Heat shock proteins and superantigenic properties of bacteria from the gastrointestinal tract of patients with Kawasaki disease. *Immunology* (2009) 128:511–20. doi: 10.1111/j.1365-2567.2009.03135.x
9. Terai M, Honda T, Yasukawa K, Higashi K, Hamada H, Kohno Y. Prognostic impact of vascular leakage in acute Kawasaki disease. *Circulation* (2003) 108:325–30. doi: 10.1161/01.CIR.0000079166.93475.5F
10. Leung DY, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* (1993) 342:1385–8. doi: 10.1016/0140-6736(93)92752-F
11. Yoshioka T, Matsutani T, Toyosaki-Maeda T, Suzuki H, Uemura S, Suzuki R, et al. Relation of streptococcal pyrogenic exotoxin C as a causative superantigen for Kawasaki disease. *Pediatr Res*. (2003) 53:403–10. doi: 10.1203/01.PDR.0000049668.54870.50
12. Konishi N, Baba K, Abe J, Maruko T, Waki K, Takeda N, et al. A case of Kawasaki disease with coronary artery aneurysms documenting *Yersinia pseudotuberculosis* infection. *Acta Pediatr*. (1997) 86:661–4. doi: 10.1111/j.1651-2227.1997.tb08952.x
13. Rowley AH. The etiology of Kawasaki disease: superantigen or conventional antigen? *Pediatr Infect Dis J*. (1999) 19:69–70. doi: 10.1097/00006454-199901000-00018
14. Choi IH, Chwae YJ, Shim WS, Kim DS, Kwon DH, Kim JD, et al. Clonal expansion of CD8+ T cells in Kawasaki disease. *J Immunol*. (1997) 159:481–6.
15. Leung DY, Gately M, Trumble A, Ferguson-Darnell B, Schlievert PM, Picker LJ. Bacterial superantigens induce T-cell expression of the skin selective homing receptor, the cutaneous lymphocyte associated antigen via stimulation of interleukin 12 production. *J Exp Med*. (1995) 181:747. doi: 10.1084/jem.181.2.747
16. Yeung RSM. Pathogenesis and treatment of Kawasaki disease. *Curr Opin Rheumatol*. (2005) 17:617–23. doi: 10.1097/01.bor.0000174184.15901.ee
17. Rowley AH, Eckerley CA, Jäck HM, Shulman ST, Baker SC. IgA plasma cell in vascular tissue of patients with Kawasaki syndrome. *J Immunol*. (1997) 159:5946–55.
18. Chang LY, Lu CY, Shao PL, Lee PI, Lin MT, Fan TY, et al. Viral infections associated with Kawasaki disease. *J Formos Med Assoc*. (2014) 113:148–54. doi: 10.1016/j.jfma.2013.12.008
19. Turnier JL, Anderson MS, Heizer HR, Jone PN, Glodé MP, Dominguez SR. Concurrent respiratory viruses and Kawasaki disease. *Pediatrics* (2015) 136:e609–14. doi: 10.1542/peds.2015-0950
20. Rowley AH, Baker SC, Orenstein JM, Shulman ST. Searching for the cause of the cause of Kawasaki disease.-cytoplasmic inclusion bodies provide new insight. *Nat Rev Microbiol*. (2008) 6:394–401. doi: 10.1038/nrmicro1853
21. Rowley AH, Baker SC, Shulman ST, Rand KH, Tretiakova MS, Perlman EJ, et al. Ultrastructural immunofluorescence, and RNA evidence support the hypothesis of a "new" virus associated with Kawasaki disease. *J Infect Dis*. (2011) 203:1021–30. doi: 10.1093/infdis/jiq136
22. Dietz SM, van Stijn D, Burgner D, Levin M, Kuipers IM, Hutten BA, et al. Dissecting Kawasaki disease: a state-of-the-art review. *Eur J Pediatr*. (2017) 176:995–1009. doi: 10.1007/s00431-017-2937-5
23. Nagata S, Yamashiro Y, Maeda M, Ohtsuka Y, Yabuta K. Immunohistochemical studies on small intestinal mucosa in Kawasaki disease. *Pediatr Res*. (1993) 33:557–63. doi: 10.1203/00006450-199306000-00004
24. Yamashiro Y, Nagata S, Oguchi S, Shimizu T. Selective increase of V beta 2 T cells in the small intestinal mucosa in Kawasaki disease. *Pediatr Res*. (1996) 39:264–6. doi: 10.1203/00006450-199602000-00013
25. Yamashiro Y, Nagata S, Ohtsuka Y, Oguchi S, Shimizu T. Microbiologic studies on the small intestine in Kawasaki disease. *Pediatr Res*. (1996) 39:622–4. doi: 10.1203/00006450-199604000-00010
26. Abe J, Kotzin BL, Jujo K, Melish ME, Glode MP, Kohsaka T, et al. Selective expansion of T cells expressing T-cell receptor variable region V beta 2 and V beta 8 in Kawasaki disease. *Proc Natl Acad Sci USA*. (1992) 89:4066–70. doi: 10.1073/pnas.89.9.4066
27. Pietra BA, De Inocencio J, Giannini EH, Hirsch R. TCR V beta family repertoire and T-cell activation markers in Kawasaki disease. *J Immunol*. (1994) 153:1881–8.
28. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (1970) 22:680–5. doi: 10.1038/227680a0
29. Kamphuis S, Kuis W, de Jager W, Teklenburg G, Massa M, Gordon G, et al. Tolerogenic immune response to novel T-cell epitopes from heat-shock protein 60 in juvenile idiopathic arthritis. *Lancet* (2005) 365:50–6. doi: 10.1016/S0140-6736(05)66827-4
30. Wick G, Perschinka H, Xu Q. Autoimmunity and atherosclerosis. *Am Heart J*. (1999) 138:S444–9. doi: 10.1016/S0002-8703(99)70272-3
31. Xu Q, Willeit J, Marosi M, Kleindienst R, Oberhollenzer F, Kiechl S, et al. Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* (1993) 341:255–9. doi: 10.1016/0140-6736(93)92613-X
32. Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J, et al. Antibodies to human heat-shock protein 60 are associated with the immune component of atherogenesis. *Circulation* (2001) 103:1071–5. doi: 10.1161/01.CIR.103.8.1071
33. Mayr M, Metzler B, Kiechl S, Willeit J, Schett G, Xu Q, et al. Endothelial cytotoxity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. *Circulation* (1999) 99:1560–6. doi: 10.1161/01.CIR.99.12.1560
34. Schett G, Xu Q, Amberger A, Van der Zee R, Recheis H, Willeit J, et al. Autoantibodies against heat shock protein 60 mediate endothelial cytotoxity. *J Clin Invest*. (1995) 96:2569–77. doi: 10.1172/JCI118320
35. Pockley AC. Heat shock proteins as regulators of immune response. *Lancet* (2003) 362:469–76. doi: 10.1016/S0140-6736(03)14075-5
36. Yokota S, Tsubaki K, Kuriyama T, Shimizu H, Ibe M, Mitsuda T, et al. Presence in Kawasaki disease of antibodies to Mycobacterial heat-shock protein 65 and autoantibodies to epitopes of human HSP65 cognate antigen. *Clin Immunol Immunopathol*. (1993) 67:163–70. doi: 10.1006/clin.1993.1060
37. Takeshita S, Kawase H, Yamamoto M, Fujisawa T, Sekine I, Yoshioka S. Increased expression of human 63-kD heat shock protein gene in Kawasaki disease determined by quantitative reverse transcription–polymerase chain reaction. *Pediatr Res*. (1994) 35:179–83. doi: 10.1203/00006450-199402000-00010
38. Sireci G, Dieli F, Salerno A. T cell recognize an immunodominant epitope of heat shock protein 65 in Kawasaki disease. *Mol Med*. (2000) 6:581–90. doi: 10.1007/BF03401796
39. Mandal K, Afzal AR, Brecker SJ, Poloniecki J, Xu Q, Jahangiri M. Association of serum soluble heat shock protein 60 with toll like receptor 4 polymorphism and severity of coronary artery disease. *Heart* (2006) 92:683–5. doi: 10.1136/hrt.2004.059170
40. Temple SE, Cheong KY, Ardlie KG, Sayer D, Waterer GW. The septic shock associated HSPA1B1267 polymorphism in?uences production of HSPA1A and HSP1B. *Intens Care Med*. (2004) 30:1761–7. doi: 10.1007/s00134-004-2359-5
41. Kumarapeli ARK, Wang X. Genetic modification of the heart: chaperones and the cytoskeleton. *J Mol Cell Cardiol*. (2004) 37:1097–109. doi: 10.1016/j.yjmcc.2004.07.004
42. Southwood S, Sidney J, Kondo A, del Guercio MF, Appella E, Hoffman S, et al. Several common HLA-DR types share largely overlapping peptide binding repertoires. *J Immunol*. (1998) 160:3363–73.
43. Park YW, Han JW, Hong YM, Ma JS, Cha SH, Kwon TC, et al. Epidemiological features of Kawasaki disease in Korea, 2006-2008. *Pediatr Int*. (2011) 53:36–9. doi: 10.1111/j.1442-200X.2010.03178.x
44. Huang WC, Huang LM, Chang IS, Chang LY, Chiang BL, Chen PJ, et al. Epidemiological features of Kawasaki disease in Taiwan, 2003-2006. *Pediatrics* (2009) 123:e401–5. doi: 10.1542/peds.2008-2187
45. 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. doi: 10.1038/ng.2007.59
46. Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. *Hum Mol Genet*. (2010) 19:2898–906. doi: 10.1093/hmg/ddq176
47. Onouchi Y. Genetics of Kawasaki disease: what we know and don't know. *Circ J*. (2012) 76:1581–6. doi: 10.1253/circj.CJ-12-0568
48. Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. *Nat Rev Rheumatol*. (2015) 11:475–82. doi: 10.1038/nrrheum.2015.54
49. Burns JC, Franco A. The immunomodulatory effects of intravenous immunoglobulin therapy in Kawasaki disease. *Expert Rev Clin Immunol*. (2015) 11:819–25. doi: 10.1586/1744666X.2015.1044980

50. Wang Y, Wang W, Gong F, Fu S, Zhang Q, Hu J, et al. Evaluation if intravenous immunoglobulin resistance and coronary lesions in relation to Th1/Th2 cytokine profile in patients with Kawasaki disease. *Arthr Rheum*. (2013) 65:805–14. doi: 10.1002/art.37815
51. Takahashi K, Oharaseki T, Yokouchi Y. Update on etio and immunopathogenesis of Kawasaki disease. *Curr Opin Rheumatol*. (2014) 26:31–6. doi: 10.1097/BOR.0000000000000010
52. Nagata S, Yamashiro Y, Fujimori M, Chiba Y, Ohtsuka Y, Shimizu T. Antimicrobial therapy using sulfamethoxazole trimethoprim for Kawasaki disease patients unresponsive to intravenous immunoglobulin. *Open J Pediatr*. (2011) 1:27–9. doi: 10.4236/ojped.2011.13007
53. Tsuji H, Nomoto K. Yakult intestinal flora-SCAN: a novel culture-independent analytical method for detection of bacteria in the bloodstream. *Ann Nutr Metab*. (2017) 71(Suppl. 1):4–10. doi: 10.1159/000479917
54. Rowley AH. Is Kawasaki disease an infectious disorder? *Int J Rheum Dis*. (2018) 21:21–5. doi: 10.1111/1756-185X.13213

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared affiliation, though no other collaboration, with the author SN at time of review.

frontiers in Pediatrics

REVIEW
published: 05 April 2019
doi: 10.3389/fped.2019.00124

The Gut Microbiota-Host Partnership as a Potential Driver of Kawasaki Syndrome

Susanna Esposito[1]*, *Ilaria Polinori*[1] and *Donato Rigante*[2,3,4]

[1] Pediatric Clinic, Department of Surgical and Biomedical Sciences, Università degli Studi di Perugia, Perugia, Italy, [2] Institute of Pediatrics, IRCCS, Rome, Italy, [3] Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy, [4] Università Cattolica Sacro Cuore, Rome, Italy

Kawasaki syndrome (KS) is a necrotizing vasculitis of small- and medium-sized vessels mostly affecting children under 5 years of age; a host of clinical and epidemiological data supports the notion that KS might result from an infectious disease. However, many efforts have failed to identify a potentially universal trigger of KS. The contribution of the intestinal microbial community—called the "microbiota"—to KS has been evaluated by an increasing number of studies, though limited to small cohorts of patients. Differences in the microbiota composition were found in children with KS, both its acute and non-acute phase, with abnormal colonization by *Streptococcus* species in the intestinal tract and a wider presence of Gram-positive cocci in jejunal biopsies. In particular, a higher number of Gram-positive cocci (of the genera *Streptococcus* and *Staphylococcus*), *Eubacterium*, *Peptostreptococcus*, and HSP60-producing Gram-negative microbes have been found in the stools of KS children, and their effects on the antigenic repertoire of specific T cells and Vβ2 T cell expansion have been assessed. Conversely, *Lactobacilli* were lacking in most children with KS compared with other febrile illnesses and healthy controls. All studies available to date have confirmed that an imbalance in the gut microbiota might indirectly interfere with the normal function of innate and adaptive immunity, and that variable microbiota interactions with environmental factors, mainly infectious agents, might selectively drive the development of KS in genetically susceptible children. Further investigations of the intestinal microflora in larger cohorts of KS patients will provide clues to disentangle the pathogenesis of this disease and probably indicate disease-modifying agents or more rational KS-specific therapies.

Keywords: Kawasaki syndrome, infection, innovative biotechnologies, microbiota, personalized medicine, child

OPEN ACCESS

Edited by:
Kyung-Yil Lee,
The Catholic University of Korea, South Korea

Reviewed by:
Valerio Iebba,
Istituto Pasteur Italia, Italy
Jeong Jin Yu,
University of Ulsan College of Medicine, South Korea

***Correspondence:**
Susanna Esposito
susanna.esposito@unimi.it

Specialty section:
This article was submitted to Pediatric Infectious Diseases, a section of the journal Frontiers in Pediatrics

Received: *03 February 2019*
Accepted: *15 March 2019*
Published: *05 April 2019*

Citation:
Esposito S, Polinori I and Rigante D (2019) The Gut Microbiota-Host Partnership as a Potential Driver of Kawasaki Syndrome. Front. Pediatr. 7:124. doi: 10.3389/fped.2019.00124

INTRODUCTION

The most insidious primary vasculitis in childhood is Kawasaki syndrome (KS), an acute multi-systemic illness which predominantly affects children under 5 years of age (1). Currently, this disorder of unknown etiology remains the main cause of acquired heart disease among children living in developed countries, where rheumatic fever has been surpassed (2, 3). This condition was originally called "mucocutaneous lymph node syndrome" by Dr. Tomisaku Kawasaki, who was its discoverer, and was thought to be a benign children's disease; nowadays, the illness has

been described worldwide in children of every ethnicity following the presence of fever persisting (at least) 5 days together with (at least) 4 of the 5 following signs: bilateral conjunctival injection, oropharyngeal inflammation, abnormalities of hands and feet, polymorphous exanthema, and non-purulent cervical lymphadenopathy, usually unilateral (4). Several years after the first description, fatalities occurred among children with KS younger than 2 years living in Japan, prompting clinicians to reconsider KS's long-term risks related to systemic and necrotizing effects on the vascular endothelium of small- and medium-sized arteries, which have been acknowledged in all guidelines related to the management of KS (5, 6). The most relevant sequelae of KS include variable degrees of damage within coronary arteries in combination with angina, myocardial infarction, ischemic cardiomyopathy, and sudden death; these complications should be preventable with a timely treatment of high-dose intravenous immunoglobulin (IVIG), which is the recommended therapeutic strategy in KS (7). Higher acute phase reactants and younger age at onset of KS are nodal points in determining, respectively, a failure in the response to IVIG and an increased occurrence of coronary artery abnormalities (8). The prediction of IVIG resistance is also crucial in KS patients, as recognizing these high-risk children should consent to start an intensified treatment protocol combined with IVIG to prevent coronary injuries (9).

KS incidence varies widely among different ethnic groups; for instance, in the United Kingdom it has stabilized and remains low at 2.8 per 100,000 population under the age of 20 years. However, general practitioners should be aware that the condition occurs throughout childhood and across the seasons, and—given the potential cardiovascular sequelae—KS should be considered in all children with persistent fever, even in older children and adolescents (10). KS is most prominently recognized in Japan, Korea, and Taiwan, reflecting increased genetic susceptibility among Asian populations. A recent study reported an incidence of ∼240 per 100,000 children under 4 years of age in Japan (11). There is still much controversy about the etiology of KS, though epidemiologic and clinical data suggest that KS might originate from an abnormal response to undisclosed infectious diseases in genetically susceptible children (12). There is no agreement whether KS-related infectious agents are of viral, bacterial or fungal origin (13), and the underlying immune mechanisms behind KS have not been completely highlighted, remaining only partially known. The absence of a proven unambiguous cause of KS has induced the scientific community to pay attention to other environmental hypothetical triggers, and in particular the composition of the resident intestinal flora as a potential contributor to KS has been evaluated by different research groups.

The main aim of this review was to analyze the relationship between the microbial community, or the "microbiota," and the overall impact of bacterial or viral infections in the potential development of KS. Scientific papers have been searched from the electronic databases of PubMed until January 2019; the retrieving words were "Kawasaki disease," "Kawasaki syndrome," "microbiota," and "microbiome"; additional reports were identified and analyzed through the specific references cited in the retrieved papers. Only papers published in English and those showing evidence-based data were included in our evaluation.

EVOLUTION OF THE MICROBIOTA IN CHILDREN

The microbiota, a microbial community of trillions of microorganisms and at least 1,000 different bacterial species, some eukaryotic fungi and viruses, and which covers every surface of the human body, plays a contributory role in many infections, immune-mediated disorders, rheumatologic diseases, and disorders of the nervous system. The microbiome, on the other hand, is the collection of the whole genome sequences of those microorganisms, consisting of more than 5,000,000 genes (14, 15). In particular, the gut microbiota is strictly linked to the chronological age of each individual and modulates host physiology and metabolism through different mechanisms.

Each stage of human life is characterized by a specific intestinal microbial composition: the microbiota that initially colonizes the fetus' intestinal tube consists of aerobic organisms such as *Enterococcus* and *Streptococcus*, is then gradually replaced by anaerobes such as *Bifidobacterium* and *Lactobacillus* and finally reaches the adult composition dominated by *Bacteroides* and Firmicutes (16, 17). The fetal microbiota is prone to be conditioned by the type of delivery; the mother's vaginal flora is a relevant source of *Lactobacillus*, *Prevotella*, and *Bifidobacterium*. Conversely, a cesarean delivery delays contact with these species, producing a similar-to-skin flora, dominated by Staphylococci (18). The feeding regimens and food supplements also play a role in modifying the resident flora; a greater complexity is normally seen in infants fed with formula, rather than in breastfed babies who have an "adult-like" structured microbiota with a population rich with Bifidobacteria, Lactobacilli, and *Bacteroides* (18). The infant gut microbiota is variable in composition over time and highly changeable during the first year of life, being influenced by specific bacteria to which a baby happens to be exposed, as shown by the resemblance of infants' stool microbial community with mothers' milk and vaginal samples (19). Thereafter, the infant's intestinal tract progresses toward an extremely dense colonization, ending with a mixture of microbes that is broadly very similar to an adult's intestine. During adulthood, the gut microbiota becomes stable and this intestinal homeostasis remains in equilibrium with the host. Food habits influence the composition of the whole intestinal microbiota, as testified by the lower prevalence of *Bacteroides* in those suffering from malnutrition and by different microbiota changes occurring in children with diet-related diseases, such as allergies and obesity (19).

Arumugam et al. identified three distinct enterotypes, namely *Bacteroides*, *Prevotella,* and *Ruminococcus*, which reflect many individual alimentary profiles: *Bacteroides* correlates to a high-fat or high-protein regimen, whereas *Prevotella* is associated with higher consumption of fibers and simple sugars (20). More recent data have consented to unify *Bacteroides* and *Ruminococcus* enterotypes due to the large similarity between the two (21). It is

well-established that early events of birth, environmental factors during infancy, sex hormones, diet, body weight, and use of antibiotics can undoubtedly differentiate the composition of the microbiota (22, 23). There are no ideal culture methods to give us a real overview of the complete intestinal flora, though molecular methods, e.g., DNA microarrays with comprehensive coverage of most bacterial taxa represented in the available database of small subunit ribosomal RNA gene sequences, should allow the characterization of most taxonomic groups of the intestinal bacteria (24, 25).

ROLE OF THE BACTERIAL FLORA IN CHILDHOOD DISEASES

The ancient symbiosis between the human gastrointestinal tract and its resident microbiota involves diverse reciprocal interactions between the microbiota itself and the host, with relevant consequences for human health and physiology. The quality of the microbial flora has an impact on the maintenance of health and also on prevention of diseases. Indeed, there are numerous roles carried out by the intestinal ecosystem, as the stimulation of angiogenesis, control of host fat storage and protection against other pathogens. In particular, the microbiota influences the formation and progress of regulation of both innate and adaptive immunity in close interaction with the intestinal mucosal immune system. The intestinal mucosa may be considered as an immunological niche as it hosts a complex immune-functional organ comprised by T cell subpopulations, neutrophils, macrophage-dendritic cells, enterocytes (that possess tight intercellular junctions) and their related anti- and pro-inflammatory cytokines as well as several other mediators of inflammation or antimicrobial peptides, defensins, and secretory immunoglobulin A (IgA) (26). The intestinal microbiota may also have direct or indirect effects on the natural course of viral infections, interacting with viral particles and leading to differences in either pathogenicity or anti-viral immune response through recruitment and activation of several T cell subpopulations (18). A large amount of data has also depicted the relevance of gut microbiota-immune system cross-talk in several diseases, and indeed an "imbalance" of the intestinal flora has been shown in patients with atopic diseases and various non-infectious diseases, including metabolic disorders, chronic inflammatory bowel disease (IBD), irritable bowel syndrome, pancreatic diseases, atherosclerosis, and rheumatoid arthritis (27, 28). Furthermore, the gut microbiota, interacting with pattern recognition receptors (PRRs), signaling receptors that can recognize molecular structures of pathogens and activate the cascade of innate immunity, plays a crucial role in maintaining the homeostasis of the innate immunity responses in the gut, and leaks in the intestinal mucosal barrier lead to the translocation of bacterial products into portal circulation which promotes systemic inflammation (29). In addition, the microbiota can maintain a segregation between intestinal mucosa and bacteria via PRRs, though pathogens might usurp innate signals to their advantage (30).

Several immunologic, metabolic and nutritional processes are normally controlled by the intestinal microbiota, and changes in the local microbial communities have been linked to chronic low-grade inflammation (31). Alexander et al. demonstrated that the microbiota has specific effects on adaptive immunity, such as the induction of regulatory effector CD4+ cells and production of cytokines and antimicrobial factors, influencing the individual response to various environmental stimuli, as seen in IBD, Crohn's disease and ulcerative colitis (32). In a recent work, Schwiertz et al. have shown microbiota changes characterized by decreased numbers of *Faecalibacterium praunsitzii* and increased numbers of *Escherichia coli* in IBD (33). Additionally, in celiac disease De Palma et al. have described a difference in the microbiota composition with a reduction of *Bifidobacterium*, *Clostridium histolyticum*, *Clostridium lituseburense*, *Faecalibacterium prausnitzii,* and an abundance of *Bacteroides* and *Prevotella* strains (34). A significantly higher biodiversity in coeliac children's duodenal mucosa was demonstrated by Schippa et al. who also highlighted that the possible pathophysiological role of such microbial differences needs further characterization (35). In addition, many immune phenomena were shown to deteriorate under the effect of changes in the microbiota, as revealed by the correlation among intestinal bacterial overgrowth, increased permeability, and development of non-alcoholic steatohepatitis (36).

Much interest has also been paid to the role of the microbiome in the development of "sterile" inflammation, and recent studies have proved that either depletion of the microbiota or changes in the diet and in the gut microbiome might lead to the improvement of inflammasome-mediated manifestations of autoinflammatory disorders, which are caused by dysregulation of specific components of innate immunity (37). These diseases can be subdivided into monogenic and multifactorial disorders, with the former being caused by mutations of genes involved in the regulation of the innate immune system and the latter by a combination of genetic background and environmental factors (38–40). Given the evidence for the role of the intestinal microbiota in the inflammatory state of IBD, atopic diseases and numerous non-infectious diseases, it has been speculated that intestinal microbial agents might also play a trigger role in the development of other inflammatory disorders which do not have a clearly defined etiology, such as KS.

UNSOLVED PROBLEMS ABOUT THE ETIOLOGY OF KAWASAKI SYNDROME

The etiology of KS remains obscure, although clinical and epidemiological features suggest a primary infectious cause. Indeed, a self-limited and generally nonrecurring illness that manifests itself by fever, rash, mucositis, conjunctival injection, and cervical adenopathy fits well with an infection. More precisely, clinical features of KS resemble some peculiar infectious diseases, such as streptococcal infections, staphylococcal toxic shock syndrome, and atypical measles (12, 13). Additionally, age distribution, winter-spring seasonality, high rates of KS in siblings, and occurrence of community

outbreaks are suggestive of a transmissible childhood disease, though many efforts with conventional bacterial and viral cultures and serological methods as well as animal inoculation studies have failed to identify a final unique infectious agent of KS (41). Reported non-infectious factors associated with KS include carpet shampoo, preexisting eczema, environmental pollution, and house dust mites (42). The higher incidence of KS in Asian populations, presence of familial clustering, and elevated risk of recurrence vs. the risk of a first episode in KS-naïve children are all strong evidence of a genetic contribution to KS susceptibility, probably in association with an infectious trigger (43, 44). However, KS does not appear easily transmittable and does not respond to any antibiotics: these characteristics should contradict a primitively infectious pathogenesis of the illness.

The most current theory about the pathogenesis of KS is that the disease results from an exaggerated immune response toward environmental stimuli occurring in a genetically susceptible child (13). The prominent role played by the immune system in KS is confirmed by the high number of studies revealing the activation of neutrophils and multiple immune cells with overproduction of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α (45, 46). Manlhiot et al. have very recently proposed a new pathogenetic model of KS in which the disease risk is determined by concurrent interacting processes: genetic susceptibility, habitual exposure to allergens, atmospheric biological particles, and infectious agents (47). A study exploring the immune responses during the acute phase of KS showed increased levels of lipopolysaccharide (LPS) bound to the surfaces of circulating neutrophils via CD14 receptor and found markedly increased levels of the soluble CD14 in the plasma (48). Furthermore, Chen et al. (49) demonstrated that NLRP3 inflammasome activation is associated with the development of coronary arteritis in a mouse model of KS, and that infusion of visfatin, a major injurious adipokine, can activate NLRP3 inflammasome and increase interleukin (IL)-1 production, leading to enhanced endothelial dysfunction. These novel molecular mechanisms of vasculitis mediated by inflammasome activation open more specifically the road to innate immunity pathways in the interpretation of KS (49, 50).

THE COMPLEX RELATIONSHIP OF KAWASAKI SYNDROME AND INFECTIONS

Unchallengeable proof that an infection is the starting point of KS is not available. Different epidemiologic studies have supported this hypothesis, based on documented infections by various microorganisms in many cases of KS. Further clues are the occurrence of KS in epidemics, higher incidence during spring and winter and early age at which the disease might be acquired, that is 6 months to 5 years (5). Striking perturbations of many immune pathways occur during the acute phase of KS, which determines a multi-cytokine cascade in the vascular endothelium with focal disruption of small- and medium-sized vessels. However, the exact key steps leading to coronary arteritis are still far from being clarified, though endothelial cell activation; prolonged start-up of neutrophils, $CD68^+$ monocyte/macrophages and $CD8^+$ lymphocytes; production of oxygen intermediates and lysosomal enzymes; and oligoclonal IgA response by plasma cells all appear to be involved (45, 46, 51–53).

The contribution of viruses to KS has been suggested by ultrastructural studies which found cytoplasmic inclusion bodies containing RNA of viral origin in the bronchial epithelia (54) as well as by studies revealing the detection of intracytoplasmic inclusion bodies containing viral proteins and nucleic acid aggregates (55, 56). Recent investigations have supported the hypothesis that immune responses in KS are oligoclonal rather than polyclonal (as found typically in superantigen-driven responses), and that IgA plasma cells play a crucial role. Indeed, higher levels of IgA have been found in the vasculature of patients with previous KS, indicating an antigen-driven response against an etiologic agent which might have a respiratory or gastrointestinal portal of entry (57).

About half of all KS patients might have one or more respiratory viruses detected by polymerase chain reaction, without any particular predominance, but a positive respiratory viral test or presence of respiratory symptoms at the time of presentation should not be used to exclude the diagnosis of KS (58). Other studies have investigated a potential relationship of KS with coronaviruses, though without finding definite proof of cause and effect (59, 60). Many viruses have been suggested to be implicated in the pathogenesis of KS, such as adenovirus, parvovirus B19, rotavirus, H1N1 influenza virus, Epstein-Barr virus, herpesvirus 6, coxsackie B3 virus, parainfluenza virus type 3, measles virus, dengue virus, human immunodeficiency virus, and varicella-zoster virus, but no significant differences emerged from case-controlled studies (61–69). One of the most difficult infections that are harder to distinguish from KS is caused by adenovirus, due to its frequent incidental detection in KS patients and frequent laboratory finding of increased inflammatory markers (70). In particular, adenovirus has been detected in 8.8 and 25% of cases with complete and incomplete KS, respectively, by Jaggi et al. (71). There are reports that have emphasized the possible relationship of cytomegalovirus (in one child from Turkey) and human bocavirus 1 (in a French cohort of 32 patients) with KS through serologic tests and molecular techniques, but this link might be only casual and misleading (72, 73).

Regarding the bacterial origin of KS, it is debated if the infectious agents might be conventional bacteria or bacteria with superantigen activity. Superantigens (SAgs) are the most powerful T cell mitogens ever discovered, with potent immunostimulatory actions that simultaneously activate the major histocompatibility complex class II molecules and T cell receptors, leading to massive activation of various immune cells. Many studies have investigated the involvement of SAgs in KS. One of the first studies showed the selective expansion of T cell receptor (TCR) Vβ2-bearing T cells in the peripheral blood of children during the acute phase of KS (74). In addition, Leung et al. isolated Staphylococci and Streptococci that produced SAgs (toxic shock syndrome toxin-1 or TSST-1, SST-1, SEB, SEC, SPEB, and SPEC) from the throat, rectum and groin in 25 of 45

patients with untreated KS (56%) in comparison with 13 of 37 control patients (35%) (75).

Matsubara et al. conducted a case-control study with a serological approach based on enzyme-linked immunosorbent assay and measured serum antibodies against staphylococcal enterotoxins, including TSST-1, and streptococcal pyrogenic exotoxin (SPE), such as SPE-A. They showed that KS patients had significant elevation of IgM antibodies against one or more of five SAgs throughout the first to the fourth disease week (76). In a further paper the same authors analyzed the studies regarding the role of SAgs produced by *Staphylococcus aureus* and *Streptococcus pyogenes* in the pathogenesis of KS, finding numerous SAgs implicated, which brought the total number of the known staphylococcal SAgs to over 20 and streptococcal SAgs to 12 (77).

The most recent work that analyzed the relationship between SAgs and KS examined different SAg derived from *Streptococcus pyogenes* in the stools of patients with KS. Stool specimens were obtained from 36 patients with KS during the acute phase and 26 age-matched healthy children. The authors examined genes related to five Sags—SPE-A, SPE-C, SPE-G, SPE-J, and TSST-1—using polymerase chain reaction; throat and stool cultures were assessed to evaluate the presence of *Streptococcus pyogenes* and *Staphylococcus aureus* in KS patients. They reported that at least one of the SAg-related genes was detected more frequently in the stools of children with KS (78). Furthermore, among 358 patients with KS, 54 developed concurrent pneumonia and 12 of these (22.2%) had high titers of anti-*Mycoplasma pneumoniae* antibody (>1:640), suggesting a potential role of *Mycoplasma pneumoniae* in KS and the importance of anti-*Mycoplasma* treatment (79).

Another study has reported a possible role of fungal infections in the development of KS in Japan, San Diego, and the island of Hawaii through an active role of tropospheric wind patterns leaving from Central Asia, in which toxins of *Candida* species are aerosolized (80). In addition, due to the observation that *Candida albicans*-derived substances, such as *Candida albicans* water-soluble fraction (CAWS), induce a coronary arteritis in mice similar to that observed in KS, Sato et al. evaluated the role of a free-β-glucan diet on CAWS-induced vasculitis and found that quality of diet might affect the progression of systemic vasculitis (81). CAWS should act as a pathogen-associated molecular pattern in mice and activate lectin pathway of complement by binding to mannose-binding lectin and inducing an acute inflammatory reaction in the vascular system (82, 83).

WHAT IS KNOWN ABOUT MICROBIOTA AND KAWASAKI SYNDROME

There are three main reasons that led us to postulate a role of the microbiota in KS. The first is the unsatisfactory microbiological data that limited the association between infections and KS and a lack of evidence of any clear relationship between one or more pathogens with this illness. Second, the most frequent bacteria or viruses associated with KS have a higher prevalence in the overall pediatric population, but only a limited number of children will develop the disease. Third, the association of genetic predisposition and environmental factors in the pathogenetic process of KS makes KS itself similar to other multifactorial diseases.

Currently, the majority of data has found that the composition of the gut microbiota in KS patients differs from healthy subjects. Lee et al. have postulated that the immune system should lose tolerance toward a part of the resident intestinal flora and that environmental factors, i.e., a Western lifestyle or improved public hygiene systems, could transform the commensal flora into a pathogen one, as observed in different gastrointestinal disorders (84).

A recent study about the resident flora and KS was focused on throat flora. Horita et al. (85) assumed that throat flora could be a reservoir of microorganisms triggering KS and, following this hypothesis, they investigated throat microorganisms of KS patients for their content and individual SAg activity. In particular, they collected throat swabs at the start of IVIG infusion in 21 KS patients and compared them with non-KS controls (displaying other febrile illnesses). The results showed no difference in the throat flora between KS and febrile controls even in the mean mitogenic activity of bacteria isolated (85).

The gastrointestinal tract could be one of the primary sites of entry of bacterial toxins in children with KS, and a perturbation in the intestinal microbiota has been linked to the disease's pathophysiology in another study by Yamashiro et al. (86) who investigated the microflora of the small intestine in 15 Japanese patients with KS. The range of bacterial species isolated from jejunal biopsies was characterized by a wider variety of Gram-positive cocci in the acute phase of KS. Notably, 5 kinds of streptococci (*Streptococcus salivarius, Streptococcus mitis, Streptococcus oralis, Streptococcus sangius,* and *Gemella haemolysans*) and 2 kinds of staphylococci (*Staphylococcus capitis* and *Staphylococcus hyicus*) were isolated only from KS patients, suggesting that some antigens inducing a delayed-type hypersensitivity reaction in the mucosa might inundate the body by breaching the barrier of the small intestinal mucosa of KS patients (86). In fact, Nagata et al. (87) investigated cell surface phenotypes of mononuclear cells and enterocytes in the jejunal mucosa of 16 Japanese patients with KS and in 10 patients with diarrhea due to cow's milk protein intolerance. Both HLA-DR+CD3+ and DR+CD4+ cells were significantly increased, and CD8+ cells significantly reduced in the lamina propria of KS patients during the acute phase compared with patients with cow's milk protein intolerance. These cell patterns returned to normal in the convalescent phase of KS. The authors concluded that a delayed-type hypersensitivity reaction was indeed present in the small intestinal mucosa of KS patients (87).

Due to the fact that the gastrointestinal tract is the largest interface between microbial factors and their host, containing the largest proportion of bacteria and the largest amount of lymphoid tissue in the body, it was hypothesized by Eladawy et al. that the intestinal milieu could be altered in children with KS, and indeed KS patients have a higher incidence of gastrointestinal symptoms and complications (88). Specifically, Takeshita et al. (89) evaluated 20 patients with KS, 20 patients with acute febrile diseases and 20 healthy children, finding that the incidence of *Lactobacilli* isolated from KS patients (2/20,

10%) was significantly lower ($p < 0.001$) than in the other cohorts. Moreover, no significant differences in the presence of *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Enterobacteriaceae*, *Bifidobacterium, Clostridium*, *Veillonella,* or *Bacteroides* were found among the three groups. Conversely, the presence of *Eubacterium* and *Peptostreptococcus* was significantly higher in KS patients than in patients with other febrile diseases ($p < 0.01$ and $p < 0.05$, respectively), though no significant differences were observed between KS patients and healthy children (89). This observation confirmed that the majority of *Lactobacillus* species are anti-inflammatory and beneficial for health, particularly during the first years of life, due to their action in protecting against colitis, reducing pro-inflammatory cytokines such as TNF-α, IL-1, or IL-6, and increasing the subsets of regulatory T cells (90). In fact, several studies have largely described the specific action of *Lactobacilli* in maintaining the epithelial homeostasis of the gut and their striking anti-inflammatory potential (91, 92).

Nagata et al. (93) also studied the role of the gut microbiota in KS pathogenesis via SAgs and heat shock proteins (Hsps) produced by gut bacteria: the authors evaluated Sags and Hsps released by microorganisms isolated from the jejunal mucosa of 19 children with KS compared with 15 age-matched healthy controls, identifying 13 strains of Gram-negative microbes from patients with KS, which produced a large amount of Hsp60, having the power to induce an over-secretion of pro-inflammatory cytokines. They also identified 18 strains of Gram-positive cocci with SAg properties which induced the expansion of Vβ2 T cells *in vitro* (93). This microbiological analysis disclosed different causative bacteria with a final common pathway of immune activation, which might contribute to the final development of KS.

In order to evaluate the differential microbiota composition of KS patients, Kinumaki et al. (94) performed a metagenomic analysis using non-culture-based methods on feces. Their study included 28 KS patients (15 males and 13 females, aged 1–114 months, with a median age of 25 months); the time of admission to the study was defined as the acute phase, while 4–6 months after the onset of KS was considered as the non-acute phase. The authors collected a total of 56 samples—28 samples each for both acute and non-acute phases. It was demonstrated that the genera *Ruminococcus*, *Roseburia,* and *Faecalibacterium* were mostly predominant during the non-acute phase, while a higher presence of *Streptococcus* spp., including *Streptococcus pneumoniae*, *pseudopneumoniae*, *mitis*, *oralis*, *gordonii*, and *sanguinis*, was detected in the fecal samples during the acute phase (94).

This novel interpretation of a disease can be shared by other conditions, as liver cirrhosis and Sjögren syndrome, in which major changes of gut microbiota with higher proportions of *Streptococcus* spp. have been demonstrated (95, 96). These findings taken as a whole suggest that many other immune-mediated disorders are likely to be connected to an abnormal bacterial colonization of the intestinal tract, with a main role for *Streptococcus* spp., and that changes in the gut microflora composition might promote systemic and extra-intestinal inflammation. Therefore, all presented studies confirm the concept that an imbalance in the gut microbiota might directly or indirectly interfere with the normal functions of the immune system, and that the interaction with other environmental factors, mainly infectious agents, might lead to the final development of KS.

TABLE 1 | Perturbation in the intestinal microbiota of patients with Kawasaki syndrome.

References	Methods	Site	No of patients	Results
Horita et al. (85)	Culture	Throat	21 patients with KS, 20 with other febrile illnesses	No difference
Takeshita et al. (89)	Culture	Gut	20 patients with KS, 20 patients with acute febrile diseases, 20 healthy children	↓ *Lactobacillus*, ↑ *Eubacterium*[a], ↑ *Peptostreptococcus*[a]
Nagata et al. (93)	Culture	Gut	19 patients with KS, 15 patients with food-sensitive enteropathy in remission	↑ Gram-negative producing hsp60, ↑ Gram-positive cocci with superantigenic properties
Kinumaki et al. (94)	Metagenomic analysis on feces	Gut	28 KS patients, 28 samples during acute-phase, 28 samples in non-acute phase	↑ *Ruminococcus*[b], ↑ *Roseburia*[b], ↑ *Faecalibacterium*[b], ↑ *Streptococcus* spp[c]

[a] *Higher in KS patients than in other febrile illness (no differences were observed between KS patients and healthy children).*
[b] *Higher in KS patients during non-acute phase of the disease.*
[c] *Higher in KS patients during acute phase of the disease.*

Table 1 shows the characteristics of the microbiota in children with KS, as emerging by the most relevant studies dedicated to this issue.

CONCLUSIVE REMARKS AND FUTURE PERSPECTIVES

As no etiologic agent has ever been accused of being directly involved in the etiology of KS, basic research evaluating the pathogenic mechanisms of this disease will probably provide better therapies and probably consent to identify the most vulnerable hosts and protect them. A potential relationship between eubiosis and the protean functions of immunity has been contemplated by different studies, and conversely a relationship should exist between dysbiosis and immunity dysfunction shown by various diseases (97). It is strengthened that heterogeneity and abnormalities in the intestinal microflora composition may trigger or contribute to the development of specific diseases, and an increasing amount of research and microbiologic observations have led to a role of the intestinal microbiota for KS to be postulated. While we are becoming convinced that a role of the microbiota is likely, we do not know exactly the basic mechanism of how the microbiota should act. The principal obstacle of today's medical literature is to understand if the microbiota modification during KS can cause the disease or if it is a mark and a consequence of the disease itself, and

if modulating the microbiota/microbiome might represent a future target of therapy in KS. This raises the hypothesis of targeting intestinal microflora in order to restore eubiosis through the rational use of antibiotics, xenobiotics, probiotics and nutrients. While multicenter trials and registries may allow us to improve the general outcome of KS, further in-depth analysis in larger cohorts of affected children will probably unravel the tangled pathogenesis of this mysterious disorder and find new targets for identifying disease-modifying agents or more specific therapies.

AUTHOR CONTRIBUTIONS

SE, IP, and DR contributed to all stages of the preparation of this manuscript, including conception and writing. All authors approved the final submitted version of the manuscript.

REFERENCES

1. Chang LY, Lu CY, Shao PL, Lee PI, Lin MT, Fan TY, et al. Viral infections associated with Kawasaki disease. *J Formos Med Assoc.* (2014) 113:148–54. doi: 10.1016/j.jfma.2013.12.008
2. Hedrich CM, Schnabel A, Hospach T. Kawasaki disease. *Front Pediatr.* (2018) 6:198. doi: 10.3389/fped.2018.00198
3. Sehgal S, Chen X, Ang JY. Epidemiology, clinical presentation, and outcomes of Kawasaki disease among hospitalized children in an inner city hospital before and after publication of the American Academy of Pediatrics/American Heart Association Guidelines for treatment of Kawasaki disease: an 11-year period. *Clin Pediatr.* (2015) 54:1283–9. doi: 10.1177/0009922815592877
4. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics.* (1974) 54:271–6.
5. Marchesi A, Tarissi de Jacobis I, Rigante D, Rimini A, Malorni W, Corsello G, et al. Kawasaki disease: guidelines of the Italian Society of Pediatrics, part I–definition, epidemiology, etiopathogenesis, clinical expression and management of the acute phase. *Ital J Pediatr.* (2018) 44:102. doi: 10.1186/s13052-018-0536-3
6. Marchesi A, Tarissi de Jacobis I, Rigante D, Rimini A, Malorni W, Corsello G, et al. Kawasaki disease: guidelines of Italian Society of Pediatrics, part II - treatment of resistant forms and cardiovascular complications, follow-up, lifestyle and prevention of cardiovascular risks. *Ital J Pediatr.* (2018) 44:103. doi: 10.1186/s13052-018-0529-2
7. De Rosa G, Pardeo M, Rigante D. Current recommendations for the pharmacologic therapy in Kawasaki syndrome and management of its cardiovascular complications. *Eur Rev Med Pharmacol Sci.* (2007) 11:301–8.
8. Rigante D, Valentini P, Rizzo D, Leo A, De Rosa G, Onesimo R, et al. Responsiveness to intravenous immunoglobulins and occurrence of coronary artery abnormalities in a single-center cohort of Italian patients with Kawasaki syndrome. *Rheumatol Int.* (2010) 30:841–6. doi: 10.1007/s00296-009-1337-1
9. Rigante D, Andreozzi L, Fastiggi M, Bracci B, Natale MF, Esposito S. Critical overview of the risk scoring systems to predict non-responsiveness to intravenous immunoglobulin in Kawasaki syndrome. *Int J Mol Sci.* (2016) 17:278. doi: 10.3390/ijms17030278
10. Hall GC, Tulloh LE, Tulloh RM. Kawasaki disease incidence in children and adolescents: an observational study in primary care. *Br J Gen Pract.* (2016) 66:271–6. doi: 10.3399/bjgp16X684325
11. Nakamura Y, Yashiro M, Uehara R, Sadakane A, Tsuboi S, Aoyama Y, et al. Epidemiologic features of Kawasaki disease in Japan: results of the 2009–10 nationwide survey. *J Epidemiol.* (2012) 22:216–21. doi: 10.2188/jea.JE20110126
12. Burgner D, Harnden A. Kawasaki disease: what is the epidemiology telling us about the aetiology? *Int J Infect Dis.* (2005) 9:185–94. doi: 10.1016/j.ijid.2005.03.002
13. Principi N, Rigante D, Esposito S. The role of infection in Kawasaki syndrome. *J Infect.* (2013) 67:1–10. doi: 10.1016/j.jinf.2013.04.004
14. Robinson CM, Pfeiffer JK. Viruses and the microbiota. *Annu Rev Virol.* (2014) 1:55–69. doi: 10.1146/annurev-virology-031413-085550
15. Sudo D, Nakamura Y. Nationwide surveys show that the incidence of recurrent Kawasaki disease in Japan has hardly changed over the last 30 years. *Acta Paediatr.* (2017) 106:796–800. doi: 10.1111/apa.13773
16. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe.* (2015) 17:690–703. doi: 10.1016/j.chom.2015.04.004
17. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci USA.* (2011) 108:4578–85. doi: 10.1073/pnas.1000081107
18. Buccigrossi V, Nicastro E, Guarino A. Functions of intestinal microflora in children. *Curr Opin Gastroenterol.* (2013) 29:31–8. doi: 10.1097/MOG.0b013e32835a3500
19. Palmer C, Bik EM, Di Giulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol.* (2007) 5:e177. doi: 10.1371/journal.pbio.0050177
20. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature.* (2011) 473:174–80. doi: 10.1038/nature09944
21. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science.* (2011) 334:105–8. doi: 10.1126/science.1208344
22. Chen J, Ryu E, Hathcock M, Ballman K, Chia N, Olson JE, et al. Impact of demographics on human gut microbial diversity in a US Midwest population. *Peer J.* (2016) 4:1514. doi: 10.7717/peerj.1514
23. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature.* (2009) 457:480–4. doi: 10.1038/nature07540
24. Dekio I, Hayashi H, Sakamoto M, Kitahara M, Nishikawa T, Suematsu M, et al. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J Med Microbiol.* (2005) 54:1231–8. doi: 10.1099/jmm.0.46075-0
25. Sakata S, Tonooka T, Ishizeki S, Takada M, Sakamoto M, Fukuyama M, et al. Culture-independent analysis of fecal microbiota in infants, with special reference to Bifidobacterium species. *FEMS Microbiol Lett.* (2005) 243:417–23. doi: 10.1016/j.femsle.2005.01.002
26. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science.* (2010) 330:1768–73. doi: 10.1126/science.1195568
27. Candela M, Rampelli S, Turroni S, Severgnini M, Consolandi C, de Bellis G, et al. Unbalance of intestinal microbiota in atopic children. *BMC Microbiol.* (2012) 12:95. doi: 10.1186/1471-2180-12-95
28. Siogren YM, Jenmalm MC, Botcher MF, Bjrksten B, Sverremark-Ekstrom E. Altered early infant gut microbiota in children developing allergy up to 5 years of age. *Clin Exp Allergy.* (2009) 39:518–26. doi: 10.1111/j.1365-2222.2008.03156.x
29. Aguilera M, Cerdà-Cuéllar M, Martínez V. Antibiotic-induced dysbiosis alters host-bacterial interactions and leads to colonic sensory and motor changes in mice. *Gut Microbes.* (2015) 6:10–23. doi: 10.4161/19490976.2014.990790
30. Davies JM, Abreu MT. Host microbe interactions in the small bowel. *Curr Opin Gastroenterol.* (2015) 31:118–23. doi: 10.1097/MOG.0000000000000143
31. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature.* (2012) 486:207–14. doi: 10.1038/nature11234
32. Alexander KL, Targan SR, Elson CO. Microbiota activation and regulation of innate and adaptative immunity. *Immunol Rev.* (2014) 260:206–20. doi: 10.1111/imr.12180
33. Schwiertz A, Jacobi M, Frick JS, Richter M, Rusch K, Köhler H. Microbiota in pediatric inflammatory bowel disease. *J Pediatr.* (2010) 157:240–4. doi: 10.1016/j.jpeds.2010.02.046

34. De Palma G, Nadal I, Medina M, Donat E, Ribes-Koninckx C, Calabuig M, et al. Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children. *BMC Microbiol.* (2010) 10:63. doi: 10.1186/1471-2180-10-63
35. Schippa S, Iebba V, Barbato M, Di Nardo G, Totino V, Checchi MP, et al. A distinctive 'microbial signature' in celiac pediatric patients. *BMC Microbiol.* (2010) 10:175. doi: 10.1186/1471-2180-10-175
36. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut.* (2001) 48:206–11. doi: 10.1136/gut.48.2.206
37. Rigante D, Vitale A, Cantarini L. Autoinflammatory diseases. In: Ragab G, Atkinson TP, Stoll ML, editors. *The Microbiome in Rheumatic Diseases and Infection.* Cham: Springer International Publishing (Springer Nature) (2018). p. 371–7. doi: 10.1007/978-3-319-79026-8_28
38. Rigante D. A systematic approach to autoinflammatory syndromes: a spelling booklet for the beginner. *Expert Rev Clin Immunol.* (2017) 13:571–97. doi: 10.1080/1744666X.2017.1280396
39. Rigante D. A developing portrait of hereditary periodic fevers in childhood. *Expert Opin Orphan Drugs.* (2018) 6:47–55. doi: 10.1080/21678707.2018.1406797
40. Rigante D. The broad-ranging panorama of systemic autoinflammatory disorders with specific focus on acute painful symptoms and hematologic manifestations in children. *Mediterr J Hematol Infect Dis.* (2018) 10:e2018067. doi: 10.4084/mjhid.2018.067
41. Falcini F, Capannini S, Rigante D. Kawasaki syndrome: an intriguing disease with numerous unsolved dilemmas. *Pediatr Rheumatol Online J.* (2011) 9:17. doi: 10.1186/1546-0096-9-17
42. Rigante D, Tarantino G, Valentini P. Non-infectious makers of Kawasaki syndrome: tangible or elusive triggers? *Immunol Res.* (2016) 64:51–4. doi: 10.1007/s12026-015-8679-4
43. Rowley AH. Is Kawasaki disease an infectious disorder? *Int J Rheum Dis.* (2018) 21:20–5. doi: 10.1111/1756-185X.13213
44. Nakamura Y. Kawasaki disease: epidemiology and the lessons from it. *Int J Rheum Dis.* (2018) 21:16–9. doi: 10.1111/1756-185X.13211
45. Burns JC, Glode MP. Kawasaki syndrome. *Lancet.* (2004) 364:533–44. doi: 10.1016/S0140-6736(04)16814-1
46. Andreozzi L, Bracci B, D'Errico F, Rigante D. A master role for neutrophils in Kawasaki syndrome. *Immunol Lett.* (2017) 184:112–4. doi: 10.1016/j.imlet.2017.02.011
47. Manlhiot C, Mueller B, O'Shea S, Majeed H, Bernknopf B, Labelle M, et al. Environmental epidemiology of Kawasaki disease: linking disease etiology, pathogenesis and global distribution. *PLoS ONE.* (2018) 13:e0191087. doi: 10.1371/journal.pone.0191087
48. Takeshita S, Tsujimoto H, Kawase H, Kawamura Y, Sekine I. Increased levels of lipopolysaccharide binding protein in plasma in children with Kawasaki disease. *Clin Diagn Lab Immunol.* (2002) 9:205–6. doi: 10.1128/CDLI.9.1.205-206.2002
49. Chen Y, Li X, Boini KM, Pitzer AL, Gulbins E, Zhang Y, et al. Endothelial NLRP3 inflammasome activation associated with lysosomal destabilization during coronary arteritis. *Biochim Biophys Acta.* (2015) 1853:396–408. doi: 10.1016/j.bbamcr.2014.11.012
50. Xia M, Boini KM, Abais JM, Xu M, Zhang Y, Li PL. Endothelial NLRP3 inflammasome activation and enhanced neointima formation in mice by adipokine visfatin. *Am J Pathol.* (2014) 184:1617–28. doi: 10.1016/j.ajpath.2014.01.032
51. Choi IH, Chwae YJ, Shim WS, Kim DS, Kwon DH, Kim JD, et al. Clonal expansion of CD8+ T cells in Kawasaki disease. *J Immunol.* (1997) 159:481–6.
52. Rowley AH, Wylie KM, Kim KY, Pink AJ, Yang A, Reindel R, et al. The transcriptional profile of coronary arteritis in Kawasaki disease. *BMC Genom.* (2015) 16:1076. doi: 10.1186/s12864-015-2323-5
53. Alexoudi I, Kanakis M, Kapsimali V, Vaiopoulos G. Kawasaki disease: current aspects on aetiopathogenesis and therapeutic management. *Autoimmun Rev.* (2011) 10:544–7. doi: 10.1016/j.autrev.2011.04.005
54. Rowley AH, Baker SC, Shulman ST, Rand KH, Tretiakova MS, Perlman EJ, et al. Ultrastructural, immunofluorescence, and RNA evidence support the hypothesis of a "new" virus associated with Kawasaki disease. *J Infect Dis.* (2011) 203:1021–30. doi: 10.1093/infdis/jiq136
55. Rowley AH, Baker SC, Shulman ST, Garcia FL, Fox LM, Kos IM, et al. RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after acute Kawasaki disease. *PLoS ONE.* (2008) 3:1582. doi: 10.1371/journal.pone.0001582
56. Rowley AH, Baker SC, Shulman ST, Fox LM, Takahashi K, Garcia FL, et al. Cytoplasmic inclusion bodies are detected by synthetic antibody in ciliated bronchial epithelium during acute Kawasaki disease. *J Infect Dis.* (2005) 192:1757–66. doi: 10.1086/497171
57. Rowley AH, Eckerley CA, Jäck HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. *J Immunol.* (1997) 159:5946–55.
58. Turnier JL, Anderson MS, Heizer HR, Jone PN, Glode MP, Dominguez SR. Concurrent respiratory viruses and Kawasaki disease. *Pediatrics.* (2015) 136:609–14. doi: 10.1542/peds.2015-0950
59. Esper F, Shapiro ED, Weibel C, Ferguson D, Landry ML, Kahn JS. Association between a novel human coronavirus and Kawasaki disease. *J Infect Dis.* (2005) 191:499–502. doi: 10.1086/428291
60. Shimizu C, Shike H, Baker SC, Garcia F, van der Hoek L, Kuijpers TW, et al. Human coronavirus NL63 is not detected in the respiratory tracts of children with acute Kawasaki disease. *J Infect Dis.* (2005) 192:1767–71. doi: 10.1086/497170
61. Chua PK, Nerurkar VR, Yu Q, Woodward CL, Melish ME, Yanagihara R. Lack of association between Kawasaki syndrome and infection with parvovirus B19, human herpes-virus 8, TT virus, GB virus C/hepatitis G virus or *Chlamydia pneumoniae*. *Pediatr Infect Dis J.* (2000) 19:477–9. doi: 10.1097/00006454-200005000-00019
62. Kanegane H, Tsuji T, Seki H, Yachie A, Yokoi T, Miyawaki T, et al. Kawasaki disease with a concomitant primary Epstein-Barr virus infection. *Acta Paediatr Jpn.* (1994) 36:713–6. doi: 10.1111/j.1442-200X.1994.tb03277.x
63. Embil JA, McFarlane ES, Murphy DM, Krause VW, Stewart HB. Adenovirus type 2 isolated from a patient with fatal Kawasaki disease. *Can Med Assoc J.* (1985) 132:1400.
64. Hagiwara K, Komura H, Kishi F, Kaji T, Yoshida T. Isolation of human herpesvirus-6 from an infant with Kawasaki disease. *Eur J Pediatr.* (1992) 152:176. doi: 10.1007/BF02072502
65. Kuijpers TW, Herweijer TJ, Scholvinck L, Wertheim-Van Dillen PM, Van De Veer EM. Kawasaki disease associated with measles virus infection in a monozygotic twin. *Pediatr Infect Dis J.* (2000) 19:350–3. doi: 10.1097/00006454-200004000-00018
66. Johnson D, Azimi P. Kawasaki disease associated with Klebsiella pneumoniae bacteremia and parainfluenza type 3 virus infection. *Pediatr Infect Dis J.* (1985) 4:100. doi: 10.1097/00006454-198501000-00024
67. Sopontammarak S, Pruekprasert P. Concomitant dengue hemorrhagic fever with Kawasaki disease. *Southeast Asian J Trop Med Public Health.* (2000) 31:190–2.
68. Joshi AV, Jones KD, Buckley AM, Coren ME, Kampmann B. Kawasaki disease coincident with influenza A H1N1/09 infection. *Pediatr Int.* (2011) 53:e1–2. doi: 10.1111/j.1442-200X.2010.03280.x
69. Rigante D, Cantarini L, Piastra M, Angelone DF, Valentini P, Pardeo M, et al. Kawasaki syndrome and concurrent Coxsackie-virus B3 infection. *Rheumatol Int.* (2012) 32:4037–40. doi: 10.1007/s00296-010-1613-0
70. Song E, Kajon AE, Wang H, Salamon D, Texter K, Ramilo O, et al. Clinical and virologic characteristics may aid distinction of acute adenovirus disease from Kawasaki disease with incidental adenovirus detection. *J Pediatr.* (2016) 170:325–30. doi: 10.1016/j.jpeds.2015.11.021
71. Jaggi P, Kajon AE, Mejias A, Ramilo O, Leber A. Human adenovirus infection in Kawasaki disease: a confounding bystander? *Clin Infect Dis.* (2013) 56:58–64. doi: 10.1093/cid/cis807
72. Usta Guc B, Cengiz N, Yildirim SV, Uslu Y. Cytomegalovirus infection in a patient with atypical Kawasaki disease. *Rheumatol Int.* (2008) 28:387–9. doi: 10.1007/s00296-007-0440-4
73. Bajolle F, Meritet JF, Rozenberg F, Chalumeau M, Bonnet D, Gendrel D, et al. Markers of a recent bocavirus infection in children with Kawasaki disease: "A year prospective study". *Pathol Biol.* (2014) 62:365–8. doi: 10.1016/j.patbio.2014.06.002

74. Yoshioka T, Matsutani T, Iwagami S, Toyosaki-Maeda T, Yutsudo T, Tsuruta Y, et al. Polyclonal expansion of TCRBV2- and TCRBV6-bearing T cells in patients with Kawasaki disease. *Immunology.* (1999) 96:465–72. doi: 10.1046/j.1365-2567.1999.00695.x
75. Leung DYM, Meissner HC, Shulman ST, Mason WH, Gerber MA, et al. Prevalence of superantigen-secreting bacteria in patients with Kawasaki disease. *J Pediatr.* (2002) 140:742–6. doi: 10.1067/mpd.2002.123664
76. Matsubara K, Fukaya T, Miwa K, Shibayama N, Nigami H, Harigaya H, et al. Development of serum IgM antibodies against superantigens of *Staphylococcus aureus* and Streptococcus pyogenes in Kawasaki disease. *Clin Exp Immunol.* (2006) 143:427–34. doi: 10.1111/j.1365-2249.2006.03015.x
77. Matsubara K, Fukaya T. The role of superantigens of group A Streptococcus and Staphylococcus aureus in Kawasaki disease. *Curr Opin Infect Dis.* (2007) 20:298–303. doi: 10.1097/QCO.0b013e3280964d8c
78. Suenaga T, Suzuki H, Shibuta S, Takeuchi T, Yoshikawa N. Detection of multiple superantigen genes in stools of patients with Kawasaki disease. *J Pediatr.* (2009) 155:266–70. doi: 10.1016/j.jpeds.2009.03.013
79. Lee MN, Cha JH, Ahn HM, Yoo JH, Kim HS, Sohn S, et al. Mycoplasma pneumoniae infection in patients with Kawasaki disease. *Korean J Pediatr.* (2011) 54:123–7. doi: 10.3345/kjp.2011.54.3.123
80. Rodo X, Curcoll R, Robinson M, Ballester J, Burns JC, Cayan DR, et al. Tropospheric winds from northeastern China carry the etiologic agent of Kawasaki disease from its source to Japan. *Proc Natl Acad Sci USA.* (2014) 111:7952–7. doi: 10.1073/pnas.1400380111
81. Sato W, Ishibashi KI, Yamanaka D, Adachi Y, Ohno N. Effects of natural and chemically defined nutrients on Candida albicans water-soluble fraction (CAWS) vasculitis in mice. *Med Mycol J.* (2017) 58:E47–62. doi: 10.3314/mmj.16-00014
82. Ishida-Okawara A, Nagi-Miura N, Oharaseki T, Takahashi K, Okumura A, Tachikawa H, et al. Neutrophil activation and arteritis induced by C. albicans water-soluble mannoprotein-beta-glucan complex (CAWS). *Exp Mol Pathol.* (2007) 82:220–6. doi: 10.1016/j.yexmp.2006.05.006
83. Nagi-Miura N, Shingo Y, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, et al. Induction of coronary arteritis with administration of CAWS (Candida albicans water-soluble fraction) depending on mouse strains. *Immunopharmacol Immunotoxicol.* (2004) 26:527–43. doi: 10.1081/IPH-200042295
84. Lee KY, Han JW, Lee JS. Kawasaki disease may be a hyperimmune reaction of genetically susceptible children to variants of normal environmental flora. *Med Hypotheses.* (2007) 69:642–51. doi: 10.1016/j.mehy.2006.12.051
85. Horita N, Yokota S, Fuse S, Takamuro M, Tomita H, Sato K, et al. The throat flora and its mitogenic activity in patients with Kawasaki disease. *Microbiol Immunol.* (2004) 48:899–903. doi: 10.1111/j.1348-0421.2004.tb03609.x
86. Yamashiro Y, Nagata S, Ohtsuka Y, Oguchi S, Shimizu T. Microbiologic studies on the small intestine in Kawasaki disease. *Pediatr Res.* (1996) 39:622–4. doi: 10.1203/00006450-199604000-00010
87. Nagata S, Yamashiro Y, Maeda M, Ohtsuka Y, Yabuta K. Immunohistochemical studies on small intestinal mucosa in Kawasakidisease. *Pediatr Res.* (1993) 33:557–63. doi: 10.1203/00006450-199306000-00004
88. Eladawy M, Dominguez SR, Anderson MS, Glodé MP. Kawasaki disease and the pediatric gastroenterologist: a diagnostic challenge. *J Pediatr Gastroenterol Nutr.* (2013) 56:297–99. doi: 10.1097/MPG.0b013e3182794432
89. Takeshita S, Kobayashi I, Kawamura Y, Tokutomi T, Sekine I. Characteristic role of intestinal microflora in Kawasaki disease. *Acta Pediatr.* (2001) 91:783–88. doi: 10.1111/j.1651-2227.2002.tb03327.x
90. Reading NC, Kasper DL. The starting lineup: key microbial players in intestinal immunity and homeostasis. *Front Microbiol.* (2011) 2:148. doi: 10.3389/fmicb.2011.00148
91. Macho Fernandez E, Valenti V, Rockel C, Hermann C, Pot B, Boneca IG, et al. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut.* (2011) 60:1050–9. doi: 10.1136/gut.2010.232918
92. Tien MT, Girardin SE, Regnault B, Le Bourhis L, Dillies MA, Coppée JY, et al. Anti-inflammatory effect of *Lactobacillus casei* on Shigella-infected human intestinal epithelial cells. *J Immunol.* (2006) 176:1228–37. doi: 10.4049/jimmunol.176.2.1228
93. Nagata S, Yamashiro Y, Ohtsuka Y, Shimizu T, Sakurai Y, Misawa S, et al. Heat shock proteins and superantigenic properties of bacteria from the gastrointestinal tract of patients with Kawasaki disease. *Immunology.* (2009) 12:511–20. doi: 10.1111/j.1365-2567.2009.03135.x
94. Kinumaki A, Sekizuka T, Hamada H, Kato K, Yamashita A, Kuroda M. Characterization of the gut microbiota of Kawasaki disease patients by metagenomic analysis. *Front Microbiol.* (2015) 6:824. doi: 10.3389/fmicb.2015.00824
95. De Paiva CS, Jones DB, Stern ME, Bian F, Moore QL, Corbiere S, et al. Altered mucosal microbiome diversity and disease severity in Sjögren syndrome. *Sci Rep.* (2016) 6:23561. doi: 10.1038/srep23561
96. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature.* (2014) 513:59–64. doi: 10.1038/nature13568
97. Iebba V, Totino V, Gagliardi A, Santangelo F, Cacciotti F, Trancassini M, et al. Eubiosis and dysbiosis: the two sides of the microbiota. *N Microbiol.* (2016) 39:1–12.

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

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 24 April 2019
doi: 10.3389/fped.2019.00158

Outcomes of Kawasaki Disease Children With Spontaneous Defervescence Within 10 Days

Ya-Chiao Hu, Hsin-Min Liu, Ming-Tai Lin, Chun-An Chen, Shuenn-Nan Chiu, Chun-Wei Lu, Luan-Yin Chang, Jou-Kou Wang and Mei-Hwan Wu*

Department of Pediatrics, National Taiwan University Hospital and Medical College, National Taiwan University, Taipei, Taiwan

Background: Kawasaki disease (KD) is one of the most common vasculitis in childhood. Intravenous γ-immunoglobulin (IVIG) is recommended to be administrated within 10 days after fever onset. However, some patients didn't have IVIG therapies because of atypical disease presentations or spontaneous defervescence. We aimed to evaluate the coronary outcomes of the KD patients who didn't receive IVIG and had defervescence within 10 days.

Methods: We retrospectively reviewed the KD patients in NTUCH between 2008 and 2015. The patients with a diagnosis of KD and had a febrile length between 5 and 10 days were enrolled. Days of fever, clinical symptoms, laboratory data at the acute stage, and series of coronary artery measurements within a minimum of 3 months after disease onset were recorded. Risk factors associated with coronary lesions 1 month after KD onset were also analyzed.

Results: Two hundred ninety-three eligible KD patients were enrolled (Male: 55.1%, mean age of onset: 1.8 years old). Thirty-seven patients had spontaneous defervescence without IVIG treatment. The incidence of coronary aneurysms at the 4th week after disease onset was higher in spontaneously defervesced KD patients than those treated with IVIG (18.9% vs. 5.1%, $p = 0.002$). Interestingly, of the 238 KD patients without coronary lesions at their acute phase, percentages of emerging coronary aneurysms became significantly higher if they didn't have IVIG therapies due to spontaneous defervescence (4/31), compared with those who received IVIG (3/208). Further analysis showed the development of coronary lesions at 1 month after disease onset was associated with younger age (<12 months old, $p = 0.024$), and leukocytosis (WBC > 17,000/cumm, $p = 0.031$).

Conclusions: 18.9% of KD patients with spontaneous defervescence had coronary aneurysms. Even without initial coronary lesions, such patients were still riskier to develop coronary aneurysms, compared with KD patients who received IVIG therapies. Such findings address the importance of refining the strategy for use of IVIG in the spontaneously defervesced KD patients within 10 days after fever onset, at least in those with age younger than 1 year and those with leukocytosis.

Keywords: Kawasaki disease, spontaneous defervescence, coronary artery lesions, immunoglobulin, risk factors

OPEN ACCESS

Edited by:
Miika Kaleva Arvonen, Independent Researcher, Kuopio, Finland

Reviewed by:
Guillermo Soza, Universidad de La Frontera, Chile
Laura Ferreras-Antolín, NHS England, United Kingdom

****Correspondence:***
Ming-Tai Lin
mingtailin@ntu.edu.tw

Specialty section:
This article was submitted to Pediatric Infectious Diseases, a section of the journal Frontiers in Pediatrics

Received: *31 December 2018*
Accepted: *03 April 2019*
Published: *24 April 2019*

Citation:
Hu Y-C, Liu H-M, Lin M-T, Chen C-A, Chiu S-N, Lu C-W, Chang L-Y, Wang J-K and Wu M-H (2019) Outcomes of Kawasaki Disease Children With Spontaneous Defervescence Within 10 Days. Front. Pediatr. 7:158. doi: 10.3389/fped.2019.00158

INTRODUCTION

Kawasaki disease (KD) is the most common pediatric systemic vasculitis. The incidence in Taiwan is the third highest globally, just lower than that in Japan and Korea (1, 2). The state-of-the-art therapy recommends the intravenous immunoglobulin (IVIG) therapy during the acute stage, which is effective to decrease the coronary complications from 20 to 5% (3). However, within 10 days, some KD patients experienced spontaneous defervescence without IVIG administration. The reasons why they didn't receive IVIG were such as incomplete presentations and consideration of the cost of medication. Although the 2012 guideline of Japanese Society of Pediatric Cardiology and Cardiac Surgery (JSPCCS) recommended clinicians may refrain from IVIG in cases of less severe KD or spontaneous defervescence, based on the considerations detailed in certain scoring systems (4), the risk-stratified management of KD patients who had spontaneous defervescence remains uncertain. Therefore, we aimed to assess the midterm coronary outcomes of Kawasaki disease children with spontaneous defervescence within 10 days and explore the risk factors associated with the development of coronary arterial lesions (CAL) in the subgroup of KD patients.

METHODS

Study Populations

This study was approved by an ethics committee of National Taiwan University Hospital. In the study, we retrospectively reviewed the institutional databases for patients with a diagnosis of Kawasaki disease at National Taiwan University Children Hospital between January 2008 and December 2015. The diagnosis of KD in this study was made based on clinical criteria for KD (5). Algorithm for the evaluation of incomplete KD patients from the 2004 American Heart Association (AHA) KD guideline (5) was applied to aid the diagnosis. We excluded patients who had defervescence <5 days and over 10 days, who received more than one dose of IVIG, who have <2 principal clinical features, who lost to follow-ups and who had alternative diagnosis eventually, such as adenovirus infection, herpetic gingivostomatitis, and scarlet fever. All patients enrolled in the study received a minimum follow-up period of 3 months in order to evaluate the coronary outcome from the acute phase to the convalescent period. We collected the patients' laboratory data at the acute and subacute phase including hemogram, the serum level of C-reactive protein, sodium, albumin, aspartate transaminase (AST), and alanine transaminase (ALT). Data of urinalysis, urine culture, viral culture, and serum anti-streptolysin O at acute phase were also collected if the patient had received the tests. All of the patients received echocardiography during the acute phase and the subacute phase after fever onset. The frequency of the following echocardiography varied according to the CAL severity. Their laboratory data collected at diagnosis and during follow-ups were reviewed.

Diagnosis Definition

Based on 2004 guideline of American Heart Association for KD (5), complete KD is diagnosed in the presence of fever for at least 5 days with at least 4 of the 5 principal clinical features. In the presence of <4 principal clinical features, the diagnosis of KD can be made when coronary artery disease is detected by 2D echocardiography. Patients who have fever ≥5 days but do not have sufficient principal clinical findings may be diagnosed with incomplete KD under the support of other clinical, laboratory and echocardiographic findings. In the current study, we defined the patients with a diagnosis of either complete or incomplete KD who had spontaneous alleviation of fever without IVIG treatment within 5–10 days after the onset of disease as "defervesced KD" (dKD).

Coronary Measurement

The coronary diameters of the left main coronary artery (LMCA), left anterior descending artery (LAD), and right coronary artery (RCA) were collected. The coronary Z score is adjusted by the body surface area and using the reference established from data of Taiwanese children (6). Coronary artery lesion (CAL) is defined as a Z score of coronary diameters $\geq +2.5$. (1) On the basis of previous research, persistent CAL for more than 4 weeks after fever onset is defined as coronary artery aneurysm (CA) in this study (7, 8). Coronary aneurysms were sub-classified based on their internal diameter as small ($+2.5 \leq Z < +5.0$), medium ($+5.0 \leq Z < 10$), and giant ($Z \geq +10.0$) (3). CALs, CAs, and the regression were diagnosed based on 2D echocardiography.

Data Analysis

Patient data were expressed as counts, percentages, medians with interquartile ranges (IQRs) for non-normal distribution data or mean with standard deviation (SD) for normal distribution data. All data analysis was conducted by SPSS statistics (version 22.0) for Windows. We used Chi-square tests and Fisher's exact tests for comparison of categorical variables. The Mann-Whitney U test and Wilcoxon rank-sum test were used for comparison of continuous variables. We set $P < 0.05$ as a statistical significance in this study.

RESULTS

Patient Characteristics

A total of 293 cases with KD diagnosis were enrolled in the study. The mean (±SD) age of the enrolled patients was 1.8±1.6 years. Thirty-seven dKD patients (i.e., spontaneous defervescence within 10 days, 12.6%) were identified. The demographics, clinical symptoms, and laboratory features for KD patients treated with IVIG and dKD patients are presented in **Table 1**. The frequency of incomplete KD in the dKD patients was 51.4%, higher than that in the IVIG-treated group (37%, $p = 0.11$)

Furthermore, of the 37 dKD patients, 18 (48.6%) patients were diagnosed as complete KD. Twelve patients had 4 principle clinical features together with a positive echocardiogram. Two patients had 4 principle clinical features and ≥3 laboratory findings described in the 2004 AHA guideline. Five patients fulfilled only 3 principle features and all of them had a positive echocardiogram. The dKD group presented less number of the five principal clinical features compared to the IVIG group [mean (±SD): 3.4 (±0.8) vs. 3.7 (±0.8), $p = 0.04$]. The dKD group

TABLE 1 | Clinical and laboratory features of KD patients with fever from 5 to 10 days.

	All ($n = 293$)	dKD patients ($n = 37$)	Patients with IVIG ($n = 256$)	*p*-value
Male gender	161 (54.9%)	20 (54.1%)	141 (55.1%)	0.91
Age ± SD [range], years	1.8 ± 1.6	2.3 ± 1.7	1.8 ± 1.6	0.06
Total febrile days ± SD	6.4 ± 1.3	6.3 ± 1.6	6.4 ± 1.3	0.44
Incomplete KD	114 (38.8%)	19 (51.4%)	96 (37.0%)	0.11
Number of principal clinical features,±SD [range]	3.7 ± 0.8	3.4 ± 0.8	3.7 ± 0.8	0.04*
Disease onset to first echocardiogram, days	6.1 ± 2.6	8.4 ± 4.5	5.7 ± 2.0	0.001*
CLINICAL FEATURES				
Conjunctival injection	269 (92.4%)	29 (78.4%)	240 (94.5%)	0.001*
Skin rash	265 (90.8%)	34 (91.9%)	231 (90.6%)	0.80
Changes in lips and oral cavity	242 (82.9%)	27 (73.0%)	215 (84.3%)	0.10
Extremities change	212 (72.4%)	29 (78.4%)	183 (71.5%)	0.38
Lymphadenopathy	87 (30.3%)	7 (18.9%)	80 (32.0%)	0.11
BCG scar reactivation	108 (53.5%)	14 (37.8%)	94 (57.0%)	0.04*
LABORATORY TEST[a]				
WBC, $\mathbf{k}/\mu L$	14.6 ± 5.1	13.2 ± 4.9	14.7 ± 5.1	0.08
Hb, g/L	11.1 ± 1.1	11.4 ± 0.8	11.0 ± 1.2	0.10
PLT, $x10^4/\mu L$	350 ± 131	399 ± 144	344 ± 128	0.02*
CRP, mg/dL	7.8 ± 6.3	4.5 ± 4.5	8.2 ± 6.3	<0.001*
AST, IU/L	79.7 ± 143.5	67.9 ± 106.0	81.2 ± 147.6	0.26
Albumin, g/L	3.8 ± 0.7	4.0 ± 0.4	3.9 ± 0.5	0.10
CORONARY OUTCOMES				
CAL at acute phase	54 (18.4%)	6 (16.2%)	48 (18.8%)	0.71
CA at the 4th week	20 (6.8%)	7 (18.9%)	13 (5.1%)	0.002*
CAL at any phase	62 (21.1%)	10 (27.0%)	52 (20.3%)	0.35

dKD: KD patients who had spontaneous alleviation of fever without IVIG treatment within 5–10 days after the onset of disease. Values are expressed as percentages (%) and [a]Mean ± SD; SD, standard deviation; BCG, bacille Calmette–Guerin; WBC, White blood cell; CRP, C-reactive protein; AST, Aspartate aminotransferase; Hb, hemoglobin; PLT, platelet; CAL, Coronary artery lesion; CA, coronary artery aneurysm.

**Statically significance is defined as $p < 0.05$.*

had a significantly lower incidence of conjunctival injection and BCG scar reactivation than the IVIG group. We analyzed the laboratory data collected at the first blood test during the acute phase, platelet count was significantly higher and serum level of C-reactive protein was lower in dKD patients, compared with KD patients with IVIG therapies (**Table 1**).

Coronary Outcomes

Of the 293 cases, 54 (18.4%) patients were found to have CAL at their acute phase and 20 (6.8%) developed CA at the 4th week after disease onset. No difference of maximal coronary Z scores or percentages of CAL were noted between IVIG group [mean (±SD):1.65 (±0.99)] and dKD group [1.49 (±0.98), $p = 0.14$] at their acute phase. At 1 month after disease onset, the maximal coronary Z score of dKD group is relatively, not significantly, larger than that of the IVIG group. However, at this time point (1 month), the dKD patients had a higher incidence of CA than the IVIG group (18.9 vs. 5.1%, $p = 0.002$) (**Figure 1**). Compared with the 256 IVIG-treated KD patients, the 37 dKD patients had their first echocardiography at an average of 8.4 days after fever onset, significantly later than the 256 IVIG-treated KD patients (5.7 days, $p = 0.001$). We did univariate analysis to explore the risk factors of coronary artery lesion at 1 month after KD onset (defined as "coronary aneurysm" in the current study) and found age, incomplete KD, IVIG treatment, changes in lips and oral cavity, white blood cell count, platelet count, and levels of albumin were associated with the development of CA (**Supplement Table 1**). To avoid interaction of the above risk factors, we conducted multivariate logistic regression and found IVIG treatment ($p = 0.008$) as well as levels of albumin ($p = 0.002$) were the only two independent risk factors for the occurrence of coronary aneurysms.

Of the 37 KD patients, 6 (16.2%), 7 (18.9%), and 2 (5.4%), respectively, had coronary dilatation at 2, 4, and 12 weeks after disease onset (**Figure 1**, **Supplement Figure 1**). The most frequently affected coronary arteries was LMCA ($n = 8$, 80%), followed by RCA ($n = 6$, 60%) On the basis of the definition of CA in the current study, 7 (18.9%) patients had CA (small, $n = 6$, median, $n = 1$). Of the 31 dKD patients without initial CAL, four patients (4/31, 12.9%, **Figure 1**) had coronary progression (to coronary aneurysms) during the first month after KD onset. Of the KD patients without CAL at their acute phase (**Figure 1**), percentages of emerging coronary aneurysms (at one month after disease onset) became significantly higher if they didn't

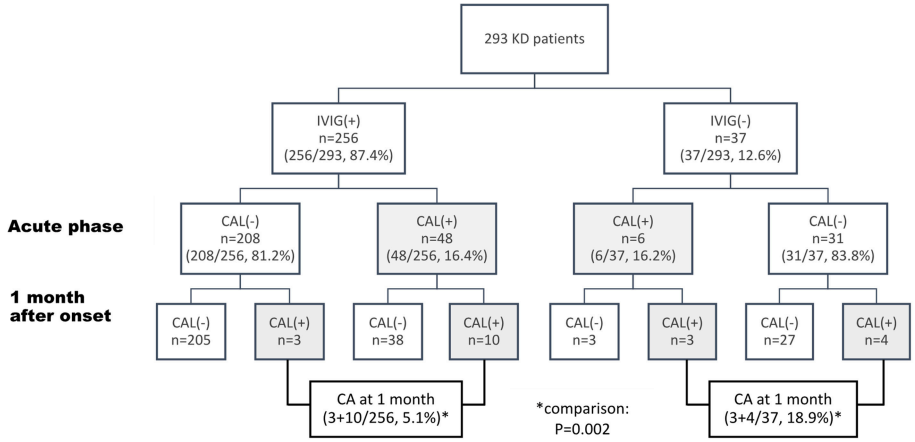

FIGURE 1 | Flow chart of management and coronary outcome of 293 patients with Kawasaki disease (KD) The data are expressed as case numbers (percentage of total patients). IVIG, intravenous immunoglobulin; CAL, coronary artery lesion, defined as Z score of coronary diameters ≥ +2.5.

have IVIG therapies due to spontaneous defervescence (4/31), compared with those who received IVIG (3/208, $p = 0.006$, **Figure 1**). Coronary aneurysms of the 4 patients regressed at 2.7, 6, 6.8, and 7.3 months after disease onset.

Risk Factors Associated With Coronary Artery Lesions in Patients With Defervescence Between 5 and 10 Days

Previous studies have demonstrated that coronary severity at 1 month after KD onset was most crucial to the late coronary outcomes. In the current study, 7 patients (18.9%, M:F = 4:3) in the dKD group had coronary lesions at 1 month after fever onset (defined as coronary aneurysms in this study). **Table 2** presents the demographic symptoms and laboratory data of dKD patients with (7) and without (30) coronary aneurysms. Univariate analysis showed the development of coronary aneurysms in the dKD group was associated with age ($p = 0.01$) and serum white blood cell count ($p = 0.03$). The percentage of younger than 1 year of age in the 7 dKD patients with coronary aneurysms was 71.4% (5/7), significantly higher than that in the 30 dKD patients without (5/30, $p = 0.009$). The 6 dKD patients with CAL at their acute phase were relatively, not significantly, risky to have coronary aneurysms, compared with 31 dKD patients without ($p = 0.07$). Furthermore, 57% dKD patients with CA (4/7) had WBC count over 17.0 kμL, which was significantly more than the dKD patient without CA (4/30, $p = 0.027$).

To avoid interaction between age and WBC count, we conducted multivariate logistic regression to identify the independent risk factors of developing coronary aneurysms in the 37 dKD patients. The results showed that age [younger than 1 year, odds ratio: 24.6, 95% confidence interval (CI) = 1.5–399, $p = 0.024$] and serum white blood cell count more than 17.0 kμL (odds ratio: 16.1, 95% CI = 1.3–198, $p = 0.031$), rather than CAL at the acute phase, were both independently associated with the occurrence of coronary aneurysms in the dKD patients.

TABLE 2 | Comparison between spontaneous defervesced KD with and without coronary artery aneurysm 1 month after disease onset.

	With CA ($n = 7$)	Without CA ($n = 30$)	*P*-value
Male gender	4 (57.1%)	16 (53.3%)	1.00
Age, years	0.7 (0.5–1.5)	2.2 (1.4–3.8)	0.011*
Age < 12 month	5 (71.4%)	5 (16.7%)	0.009*
Age < 24 month	7 (100%)	15 (50%)	0.028*
Total febrile days	6.5 (5.0–7.0)	6.0 (5.3–7.8)	0.227
Incomplete KD	5 (71.4%)	12 (40.0%)	0.405
Number of principal clinical features	3 (3–3)	4 (2–5)	0.556
CAL at acute phase	3 (42.9%)	3 (10.0%)	0.07
Laboratory test			
WBC, **k/μL**	17.84 (15.32–21.82)	12.10 (9.36–14.89)	0.033*
Hb, g/L	10.8 (10.5–10.9)	11.4 (10.9–12.1)	0.173
Hb < 11 g/L	4 (66.7%)	8 (28.6%)	0.154
PLT, x**$10^4/\mu L$**	534 (413–616)	408 (276–479)	0.091
CRP, mg/dL	5.04 (4.14–5.23)	1.98 (1.15–6.74)	0.408

Values are expressed as medians (IQRs) or number and percentages (%); WBC, White blood cell; Seg, Neutrophil segment; CRP, C-reactive protein; AST, Aspartate aminotransferase; Hb, hemoglobin; PLT, platelet.
**Statically significance is defined as $p < 0.05$.*

DISCUSSION

IVIG therapy remained the standard treatment for KD, which can effectively reduce the risk of CAL development. However, in the cases with spontaneous defervescence, treatment guidelines were still uncertain because of short of well-established data of their clinical outcomes. In this retrospective cohort, we found that 12.6% of KD patients had spontaneous defervescence

within 10 days and 18.9% of such patients (dKD) suffered from coronary aneurysms, significantly higher than the percentages of coronary aneurysms in the IVIG group (5.1%, $p = 0.002$, **Table 1**, **Figure 1**). Such findings reinforce the importance of IVIG in the treatment of KD. We also demonstrated, in the KD patients without CAL at their acute phase, percentages of emerging coronary aneurysms (at 1 month after disease onset) became significantly higher if they didn't have IVIG therapies due to spontaneous defervescence (4/31), compared with those who received IVIG (3/208). Such observation had never been described before and drove us to look for the potential risk factors of developing coronary aneurysms in the dKD patients.

We demonstrated that, among the 37 dKD patients, younger age (<1 year old) and leukocytosis were associated with the development of coronary aneurysms. Downie et al. reported that males, age <1-year-old and higher platelet count were associated with increased odds of CA formation for patients with delayed or no treatment compared to KD patients with prompt IVIG (9). Younger KD patients were thought of being less likely to have a complete presentation of KD and also suffered from an increased risk of CAL (10–12). For example, Salgado et al. showed percentages of CAL and incomplete KD were significantly higher in the KD patients younger than 6 months old, compared with those were older. Moreover, 18.6% of patients <6 months old with normal echocardiogram initially developed CAL within 8 weeks of diagnosis (13). Our data presented similar findings and younger age remained an important risk factor of CAL even though the patient had spontaneous defervescence. Taken the above together, for the KD patients younger than 1 year old, we may recommend the use of IVIG even if they have defervescence without IVIG therapy because of increased risk of CAL.

Spontaneous defervescence without IVIG is not new for the KD patients. Depending on the definition of spontaneous defervescence, the percentages ranged from 7.3 to 20.9% (7, 14, 15). Takahashi et al enrolled patients who had spontaneously defervescence within 7 days, rather than 10 days as our cohort, and their results showed 7.3% of 968 KD patients had fever persistent < 7 days without an infusion of IVIG. If we applied the same enrollment criteria as Takahashi et al. (14), to our current cohort, the percentage of spontaneous defervescence would decrease from 12.6 to 10.5%. Spontaneous defervescence in KD patients usually causes hesitation of physicians and parents on the use of IVIG. However, Takahashi et al. showed 11.2% of KD patients with spontaneous defervescence within 7 days after disease onset may have recurrent fever 3–7 days later and predispose to develop coronary artery lesions (14). The current study further innovatively points out, even without initial CAL and recurrence of fever, such subgroup of KD patients still carried a significantly higher risk of late development of coronary aneurysms, compared with those had IVIG therapies (4/31 vs. 3/208, $p = 0.006$). Both age (younger than 1 year) and leukocytosis were independently associated with the development of coronary aneurysms in the dKD patients. In summary, it's time to refine the strategy for use of IVIG in the spontaneously defervesced KD patients within 10 days after fever onset, at least in those with age younger than 1 year and those with leukocytosis.

Studies in the 1990s (16, 17) demonstrated, in the KD patients without IVIG treatment, levels of TNF alpha and IL-2 continued increasing even at the second or third week after fever onset. Until 2–3 months later, levels of the two cytokines decreased gradually to the normal ranges. TNF alpha and IL-2 were two of the key inflammatory cytokines in the pathogenesis of KD and associated with recruitment of immune cell population, T cell activation, and CAL development (18). Together the above, for the KD patients with spontaneous resolution of fever within 10 days, delayed normalization or even increase in levels of cytokines, like TNF alpha and IL-2, may accompany higher risk to the development of coronary lesions.

There were several limitations in this study. First, there was selection bias especially when we enrolled the KD patients with atypical disease presentations. To minimize the bias, we used the evaluation algorithm for suspected incomplete KD according to AHA guideline (3) and traced their clinical presentation as well as laboratory data in the subacute phase to aid diagnosis. Second, the number of dKD patients was small which made the statistical analysis limited. Third, information bias might exist, since the ultrasound technicians were not blind to tentative or previous diagnosis. Finally, because of the observational nature of our study, large-scaled prospective studies are necessary to determine the efficacy and necessity of IVIG in dKD patients who had higher risk to develop coronary abnormalities.

CONCLUSIONS

18.9% of KD patients with spontaneous defervescence had coronary aneurysms. Even without initial coronary lesions, such patients were still riskier to develop coronary aneurysms, compared with KD patients who received IVIG therapies. Such findings address the importance of refining the strategy for use of IVIG in the spontaneously defervesced KD patients within 10 days after fever onset, at least in those with age younger than 1 year and those with leukocytosis.

AUTHOR CONTRIBUTIONS

M-TL and M-HW contributed to the conception and design of the study. H-ML and L-YC organized the database. Y-CH and M-TL performed the statistical analysis. Y-CH wrote the first draft of the manuscript. C-AC, S-NC, C-WL, and J-KW took care of patients and collected data. M-TL had full data access and is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to manuscript revision, read 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.2019.00158/full#supplementary-material

REFERENCES

1. Newburger JW, Takahashi M, Burns JC. Kawasaki disease. *J Am Coll Cardiol.* (2016) 67:1738–49. doi: 10.1016/j.jacc.2015.12.073
2. Huang WC, Huang LM, Chang IS, Chang LY, Chiang BL, Chen PJ, et al. Epidemiologic features of Kawasaki disease in Taiwan, 2003-2006. *Pediatrics.* (2009) 123:e401–5. doi: 10.1542/peds.2008-2187
3. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. *Circulation.* (2017) 135:e927–99. doi: 10.1161/CIR.0000000000000484
4. Research Committee of the Japanese Society of Pediatric Cardiology, Cardiac Surgery Committee for Development of Guidelines for Medical Treatment of Acute Kawasaki Disease. Guidelines for medical treatment of acute Kawasaki disease: report of the Research Committee of the Japanese Society of Pediatric Cardiology and Cardiac Surgery (2012 revised version). *Pediatr Int.* (2014) 56:135–58. doi: 10.1111/ped.12317
5. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, treatment, and long-term management of Kawasaki disease. *Circulation.* (2004) 110:2747–71. doi: 10.1161/01.CIR.0000145143.19711.78
6. Lin MT, Chang CH, Hsieh WC, Chang CE, Chang YM, Chen YC, et al. Coronary diameters in taiwanese children younger than 6 years old: Z-score regression equations derived from body surface area. *Acta Cardiol Sin.* (2014) 30:266–73.
7. Lin MT, Sun LC, Wu ET, Wang JK, Lue HC, Wu MH. Acute and late coronary outcomes in 1073 patients with Kawasaki disease with and without intravenous gamma-immunoglobulin therapy. *Arch Dis Child.* (2015) 100:542–7. doi: 10.1136/archdischild-2014-306427
8. Chih WL, Wu PY, Sun LC, Lin MT, Wang JK, Wu MH. Progressive coronary dilatation predicts worse outcome in Kawasaki disease. *J Pediatr.* (2016) 171:78–82 e71. doi: 10.1016/j.jpeds.2015.12.076
9. Downie ML, Manlhiot C, Collins TH, Chahal N, Yeung RSM, McCrindle BW. Factors associated with development of coronary artery aneurysms after Kawasaki disease are similar for those treated promptly and those with delayed or no treatment. *Int J Cardiol.* (2017) 236:157–61. doi: 10.1016/j.ijcard.2017.01.068
10. Rosenfeld EA, Corydon KE, Shulman ST. Kawasaki disease in infants less than one year of age. *J Pediatr.* (1995) 126:524–9. doi: 10.1016/S0022-3476(95)70344-6
11. Song D, Yeo Y, Ha K, Jang G, Lee J, Lee K, et al. Risk factors for Kawasaki disease-associated coronary abnormalities differ depending on age. *Eur J Pediatr.* (2009) 168:1315–21. doi: 10.1007/s00431-009-0925-0
12. Satoh K, Wakejima Y, Gau M, Kiguchi T, Matsuda N, Takasawa R, et al. Risk of coronary artery lesions in young infants with Kawasaki disease: need for a new diagnostic method. *Int J Rheum Dis.* (2018) 21:746–54. doi: 10.1111/1756-185X.13223
13. Salgado AP, Ashouri N, Berry EK, Sun X, Jain S, Burns JC, et al. High risk of coronary artery aneurysms in infants younger than 6 months of age with Kawasaki disease. *J Pediatr.* (2017) 185:112–6 e111. doi: 10.1016/j.jpeds.2017.03.025
14. Takahashi T, Sakakibara H, Morikawa Y, Miura M. Development of coronary artery lesions in indolent Kawasaki disease following initial spontaneous defervescence: a retrospective cohort study. *Pediatr Rheumatol Online J.* (2015) 13:44. doi: 10.1186/s12969-015-0042-8
15. Liu MY, Liu HM, Wu CH, Chang CH, Huang GJ, Chen CA, et al. Risk factors and implications of progressive coronary dilatation in children with Kawasaki disease. *BMC Pediatr.* (2017) 17:139. doi: 10.1186/s12887-017-0895-8
16. Lin CY, Lin CC, Hwang B, Chiang BN. The changes of interleukin-2, tumor necrotic factor and gamma-interferon production among patients with Kawasaki disease. *Eur J Pediatr.* (1991) 150:179–82. doi: 10.1007/BF01963561
17. Lin CY, Lin CC, Hwang B, Chiang B. Serial changes of serum interleukin-6, interleukin-8, and tumor necrosis factor alpha among patients with Kawasaki disease. *J Pediatr.* (1992) 121:924–6. doi: 10.1016/S0022-3476(05)80343-9
18. Wang Y, Wang W, Gong F, Fu S, Zhang Q, Hu J, et al. Evaluation of intravenous immunoglobulin resistance and coronary artery lesions in relation to Th1/Th2 cytokine profiles in patients with Kawasaki disease. *Arthritis Rheum.* (2013) 65:805–14. doi: 10.1002/art.37815

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

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 21 May 2019
doi: 10.3389/fped.2019.00202

A Presumed Etiology of Kawasaki Disease Based on Epidemiological Comparison With Infectious or Immune-Mediated Diseases

Jung-Woo Rhim[1,2], *Hyun Mi Kang*[1,2], *Ji-Whan Han*[1] *and Kyung-Yil Lee*[1,2]*

[1] Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, South Korea, [2] Department of Pediatrics, Daejeon St. Mary's Hospital, The Catholic University of Korea, Daejeon, South Korea

Background: Kawasaki disease (KD) may be associated with infection of unknown pathogen(s). For predicting of the etiology of KD, we evaluated epidemiological characteristics in KD, common infectious diseases and immune-mediated diseases in childhood.

Methods: We respectively, reviewed the data of patients with KD, influenza, aseptic meningitis, exanthem subitum (ES), *Mycoplasma pneumoniae* (MP) pneumonia, acute pyelonephritis (APN), Henoch-Schönlein purpura (HSP), acute poststreptococcal glomerulonephritis (APSGN), and childhood asthma. We compared and interpreted epidemiological data across the groups.

Results: In age distribution, KD, APN, and ES showed a similar pattern in that majority of patients were infants or young children, and other diseases showed a relatively even age-distribution which had a peak age, mainly 5–6 years, with bell-shape patterns. In annual-case pattern, there were epidemic years in aseptic meningitis and MP pneumonia, and the fluctuated annual cases were seen in other diseases. The trends of decreasing cases were seen in APSGN, HSP, and childhood asthma in recent years. In seasonal frequency, influenza or aseptic meningitis occurred in mainly winter or summer season, respectively. HSP and APSGN cases had less in summer, and KD, APN, and ES showed relatively even occurrence throughout a year without significant seasonal variations.

Conclusions: Our results suggest that KD agents may be associated with normal flora that are influenced by environmental changes, since pathogens of APN and ES could be regarded as normal flora that originate from the host itself or ubiquitously existing human reservoirs.

Keywords: Kawasaki disease, etiology, epidemiology, acute pyelonephritis, exanthem subitum, microbiota

OPEN ACCESS

Edited by:
Claudio Pignata,
University of Naples Federico II, Italy

Reviewed by:
Elma Inés Nievas,
Independent Researcher, Mendoza, Argentina
Piero Pavone,
Università degli Studi di Catania, Italy

***Correspondence:**
Kyung-Yil Lee
leekyungyil@catholic.ac.kr

Specialty section:
This article was submitted to Pediatric Immunology, a section of the journal Frontiers in Pediatrics

Received: *30 October 2018*
Accepted: *30 April 2019*
Published: *21 May 2019*

Citation:
Rhim J-W, Kang HM, Han J-W and Lee K-Y (2019) A Presumed Etiology of Kawasaki Disease Based on Epidemiological Comparison With Infectious or Immune-Mediated Diseases. Front. Pediatr. 7:202. doi: 10.3389/fped.2019.00202

INTRODUCTION

Kawasaki disease (KD) disease was a novel disease that appeared in East Asian countries in the order of Japan, South Korea, Taiwan, and China with a 5–10 year time-gap (1–4). After the first case report in the 1960s in Japan, KD has been reported in over 60 countries around the world (5). However, the incidence of KD is different across various populations; currently, the incidence in

Western countries is lower by one tenth to one twentieth compared to East Asian countries, and has plateaued during recent decades. Whereas, the number of KD patients in East Asian countries has slowly and steadily increased after its first emergence (6). In these countries, KD occurs mostly in children between 6 months and 4 years of age, and has become a nationwide endemic disease within 2 decades after its first appearance. These findings suggest that KD spreads slowly to other regions within a nation and neighboring countries, which is contrasting to common infectious disease epidemics. KD pathogen(s) should satisfy with the epidemiological characteristics of KD.

The clinical manifestations of Kawasaki disease share similarities in some aspects of viral diseases, bacterial diseases, or infection-related immune mediated diseases such as systemic juvenile idiopathic rheumatoid arthritis or acute rheumatic fever (ARF). However, extensive studies searching for the etiologic agent(s) have turned up as failures until the present time (7). Common childhood infectious diseases and infection-related immune-mediated diseases, including KD, childhood idiopathic thrombocytopenia, and Henoch-Schönlein purpura (HSP) have low prevalence in older children and adults. Thus, these childhood diseases have been hypothesized to be associated with infections caused by unknown pathogens (8, 9). Since KD is an acute self-limiting systemic inflammation that involves multiple organs, it has been proposed that there are etiologic substances that induce systemic inflammation and target cell injuries, including coronary artery lesions (CALs), during or after the infection with KD pathogen (s) (10). Also, it has been proposed that KD pathogens may be a species of the host's normal flora based on clinical and epidemiological characteristics of KD (11). The epidemiological characteristics of a disease may aid in predicting the etiologic agent(s) of the disease. The identification of KD etiology is essential for understanding the disease, and the development of diagnostic tools and adequate treatment modalities.

In this study, to predict the etiology of KD, we evaluated and compared epidemiological characteristics between KD and common infectious diseases or immune-mediated diseases in children. After evaluating epidemiological parameters focused on age distribution, annual-case pattern and monthly-case pattern, we found that the epidemiological profiles in KD were similar to those in acute pyelonephritis (APN) or exanthema subitum (ES). We discussed the implications of these findings in KD epidemiology in Korea.

MATERIALS AND METHODS

The subjects of this study were collected from the patients (0–15 years of age) who were admitted at The Catholic University of Korea Daejeon St. Mary's Hospital from January 1987 to December 2016. We evaluated the epidemiological characteristics of patients diagnosed with *Mycoplasma pneumoniae* (MP) pneumonia, influenza, aseptic meningitis, APN, ES, acute poststreptococcal glomerulonephritis (APSGN), HSP, childhood asthma (or recurrent wheezing episode), and KD. As an exception, the subjects diagnosed with influenza in this study were selected from outpatients who were positive for influenza in rapid diagnostic testing during the winter of influenza seasons. The diagnosis or selection criteria in each disease were referenced from other publications (12–20). Although the study period and the number of patients in each disease were not identical, we reevaluated the data that were used for previously published papers or collected new data for some diseases such as ES and HSP. Age, sex, age distribution pattern, annual-case pattern and monthly-case pattern were analyzed in each disease. For mean age, <12 months of age was regarded as 0 years of age for statistical analyses, except ES. In age distribution pattern, we focused on age predilection in infancy and younger children. In annual-case pattern, we searched whether the pattern shown cyclic epidemics during the study periods. As for seasonal variation, the disease was regarded as having seasonality when the number of seasonal cases showed over 10 or 15% difference in the number of cases between the highest season and the lowest season.

Ethics Statement

The written informed consents were obtained from the parents/caregivers of all children for the medical records to be used in this study at time of admission. The study was approved by the Institutional Review Board of The Catholic University of Korea Daejeon St. Mary's Hospital (DC18RESI0100).

RESULTS

A total of 7,832 patients diagnosed with 9 diseases, including KD, 5 infectious diseases, and 3 immune-mediated diseases were evaluated. The study period in the majority of diseases was over 10 years; for infectious diseases, MP pneumonia (2003–2012, $n = 779$) (12), influenza (2010–2017, $n = 2{,}163$) [(13, 14), unpublished observation], aseptic meningitis (1987–2003, $n = 2{,}201$) (15), APN (2005–2015, $n = 320$) (16) and ES (2005–2016, $n = 429$), and as for infection-related immune diseases, APSGN (1987–2013, $n = 99$) (17), HSP (1987–2015, $n = 515$) (unpublished observation) and childhood asthma (or recurrent wheezing episode) (2003–2014, $n = 384$) (18), and KD (1987–2016, $n = 942$) (19, 20) (**Table 1**).

Age, Sex, and Age Distribution in KD and Other Diseases

The male-to-female (M:F) ratio, mean age, and peak ages are shown in **Table 1**.

Male predominance was observed in all the diseases except ES (0.95:1), but the M:F ratios were somewhat different across the diseases. The highest M:F ratio was seen in APSGN (2.3:1), and the lower M:F ratios were seen in ES, MP pneumonia and influenza (1.1:1, respectively), and KD showed 1.7:1 ratio in this series. The lowest mean age was noted in order of ES (mean 1 year of age), APN (1.5 years), and KD (2.2 years) and the higher mean age was noted in APSGN (8.3 years) and HSP (6.5 years). In age distribution, KD, APN, and ES showed a similar pattern in that the majority of patients were infants and young children (0–4 years), and after this age period the prevalence decreased

TABLE 1 | Demographic findings in KD and other diseases (0–15 years of age).

Diseases	Study period	No. of subjects	Mean cases/y	M:F ratio	Mean age (y)	Peak ages (y)
Kawasaki disease	1987–2016	942	31	1.7:1	2.2 ± 1.6	1
Mycoplasma pneumonia	2003–2012	779	78	1.1:1	5.0 ± 3.2	4
Influenza	2010–2018	2,163*	270	1.1:1	5.4 ± 3.8	3–5
Aseptic meningitis	1987–1998	2,201	115	2:1	6.0 ± 3.9	4–6
Acute pyelonephritis	2005–2015	320	29	1.4:1	1.5 ± 3.4	0
Exanthem subitum	2005–2016	429	36	1:1	1.0 ± 0.5	0
APSGN	1987–2014	99	4	2.3:1	8.3 ± 2.7	7–9
Henoch-Shönlein purpura	1987–2015	515	18	1.2:1	6.5 ± 3.0	5
Childhood asthma	2003–2014	384	32	1.2:1	5.6 ± 3.5	2–4

Outpatients; APSGN, acute poststreptoccocal glomerulonephritis.

dramatically. There were only a few patients >3 years of age in ES and a few patients >5 years in KD, however there was relatively an even distribution with female predominance after >2 years in APN. A bell-shape distribution pattern with a peak prevalence, mainly in the 4–6 years age range was observed in other diseases, however peak ages were slightly different across the diseases (**Figure 1**). There was a trend where the peak ages were changed in the epidemic diseases such as MP pneumonia and influenza over time [(12), unpublished observation].

Annual-Case Patterns in KD and Other Diseases

Cyclic epidemics caused by possibly new strains of pathogens, such as macrolide-resistant strains were noted in MP pneumonia and in aseptic meningitis caused by mainly enteroviruses; all had similar patterns with nationwide epidemics or outbreaks in Korea (12, 15). Influenza occurred every winter and early spring, but the number of cases and peak epidemic months were different in 2009 pandemic and seasonal influenza, across seasonal influenza after 2009 pandemic [(14), unpublished observation]. Relatively an even, but fluctuating annual cases were noted in KD, APN, ES, HSP, and childhood asthma, except APSGN (**Figure 2**). The mean annual cases in each disease are shown **Table 1**, and the results may be helpful to estimate the incidence of each disease in our city, since nationwide KD epidemiological studies in Korea have been performed every 3 years (21, 22). There was a trend of decreasing number of cases in the recent years or decades in APSGN, HSP and childhood asthma, whereas an increasing trend was noted in APN, compared to the past years or decades (**Figure 2**).

Monthly or Seasonal-Case Pattern in KD and Other Diseases

A higher number of cases were seen in the fall and winter in MP pneumonia. Aseptic meningitis was noted mainly during the summer whereas influenza mainly during winter and early spring seasons. There was a trend showing a lower number of cases in the summer in HSP, and a higher number of cases in the winter for APSGN. A higher number of cases were seen during the fall and spring for childhood asthma. Although KD affected more patients during summer than any other season in this series, KD had no difference in seasonal variation according to the definition of the seasonality. Also, no seasonality was seen in APN and ES (**Table 2** and **Figure 3**).

DISCUSSION

It is hypothesized that KD may have an etiologic agent(s), although many studies searching for the etiology of KD have failed. Etiologic agents in infectious diseases originate from an external source and invade into the host. However, at present time, they could be classified as exogenous pathogens and the endogenous pathogens; the former is generally accepted as agents that are newly introduced to humans from other animal species or other places, whereas the latter originates from the host in the forms of normal flora (or commensals) or those becoming a normal flora from initially being an exogenous pathogen. For examples, measles in immune-innocent populations in the past and acquired immunodeficiency syndrome (AIDS) were caused by new external pathogens. On the other hand, the pathogens causing APN or acute otitis media, such as *E. coli* or *Streptococcus pneumoniae,* could be categorized as endogenous pathogens. It is well-known that a newly introduced disease by an exogenous pathogen has affected all aged persons in a population and spread to other populations in relatively a short-time period of time after its first emergence. Over time, infected persons and groups have immunity to the pathogens, and young children and infants would remain susceptible, especially during cyclic epidemic viral infections. Accordingly, epidemiologic characteristics of infectious diseases or infection-related diseases in the populations at a given time may differ.

In the present study, we found that epidemiological characteristics in KD were most similar to those in APN or ES among the 8 evaluated diseases. In age distribution, these 3 diseases had an age predilection in infancy and young children; 79.7% of patients in APN, 95.8% in ES, and 50.1% in KD were 0–1 years of age, and 87.5% of patients in APN, near 100% in ES, and 91.7% in KD were 0–4 years of age. In the view of epidemiological and clinical aspects, an infected infant with pathogens from these diseases are difficult to disperse the pathogens to other infants. Other diseases showed a relatively even age-distribution

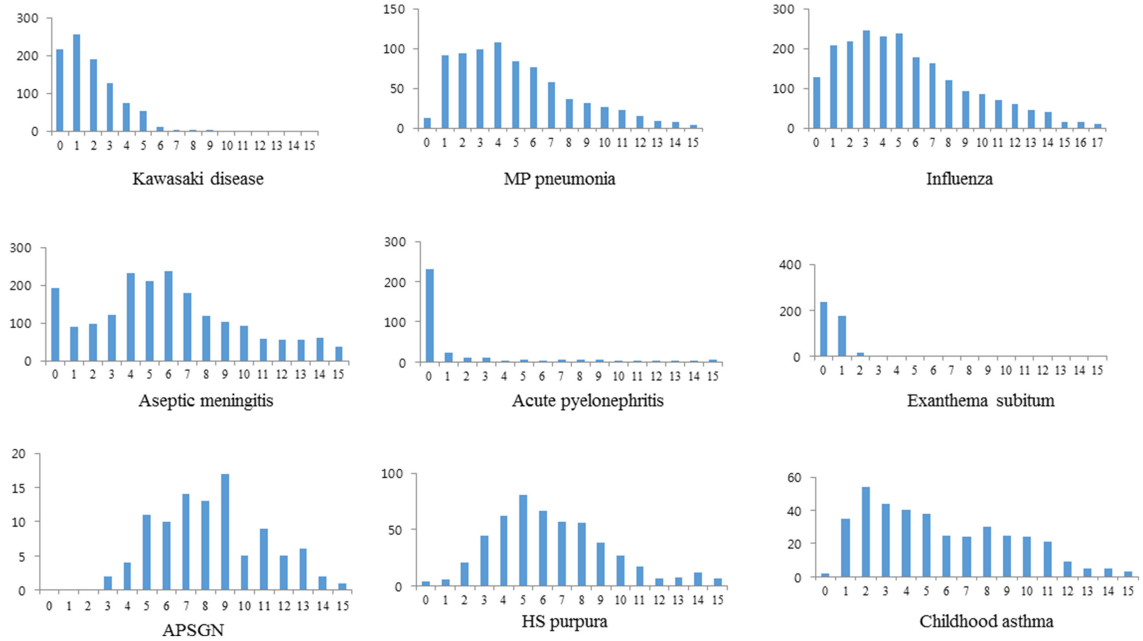

FIGURE 1 | Age distribution in Kawasaki disease and other infectious or infection-related immune-mediated diseases.

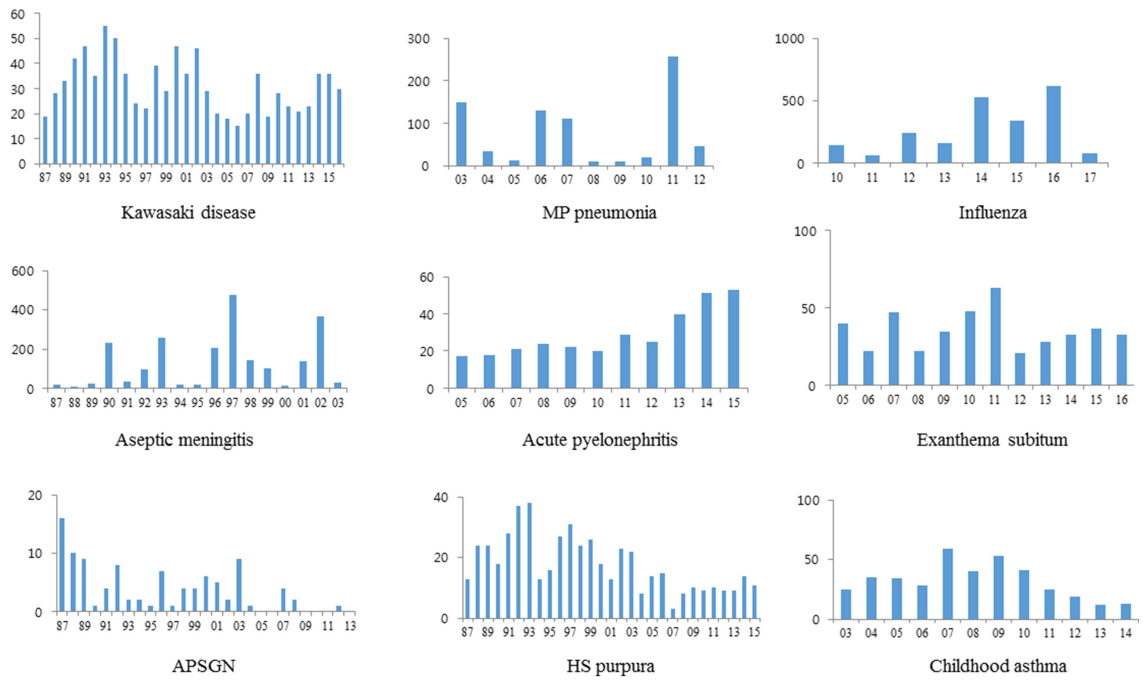

FIGURE 2 | Annual cases in KD and other diseases.

throughout childhood, though peak ages were slightly different across the diseases. This suggests that younger children may have less chance on exposure to the pathogens and older children and young adult groups may have immunity or tolerability to the pathogens causing the diseases.

In the annual-case pattern, cyclic epidemics were noted in MP pneumonia and aseptic meningitis, and marked fluctuated cases with a different peak week were noted in influenza. The annual-case pattern in KD was relatively even, but fluctuations in annual cases were noted as well as in APSGN, HSP and childhood

TABLE 2 | Seasonality in KD and other diseases.

Diseases	Spring (%)	Summer (%)	Fall (%)	Winter (%)	Seasonality
Kawasaki disease	22.9	27.7	23.1	26.2	No seasonality
Mycoplasma pneumonia	11.2	19.1	48.4	21.3	Fall predominance
Influenza	45.3	0.2	0.1	54.4	Spring, winter
Aseptic meningitis	22.2	63.5	12.3	1.9	Summer predominance
Acute pyelonephritis	26.9	27.8	23.1	22.2	No seasonality
Exanthem subitum	28.4	31.7	18.6	21.8	No seasonality
APSGN	21.2	12.1	30.3	36.4	Winter, fall
Henoch-Shönlein purpura	31.1	13.6	29.1	26.2	Low in summer
Childhood asthma	26.8	13.5	43	16.7	Fall, Spring

APSGN, acute poststreptococcal glomerulonephritis.

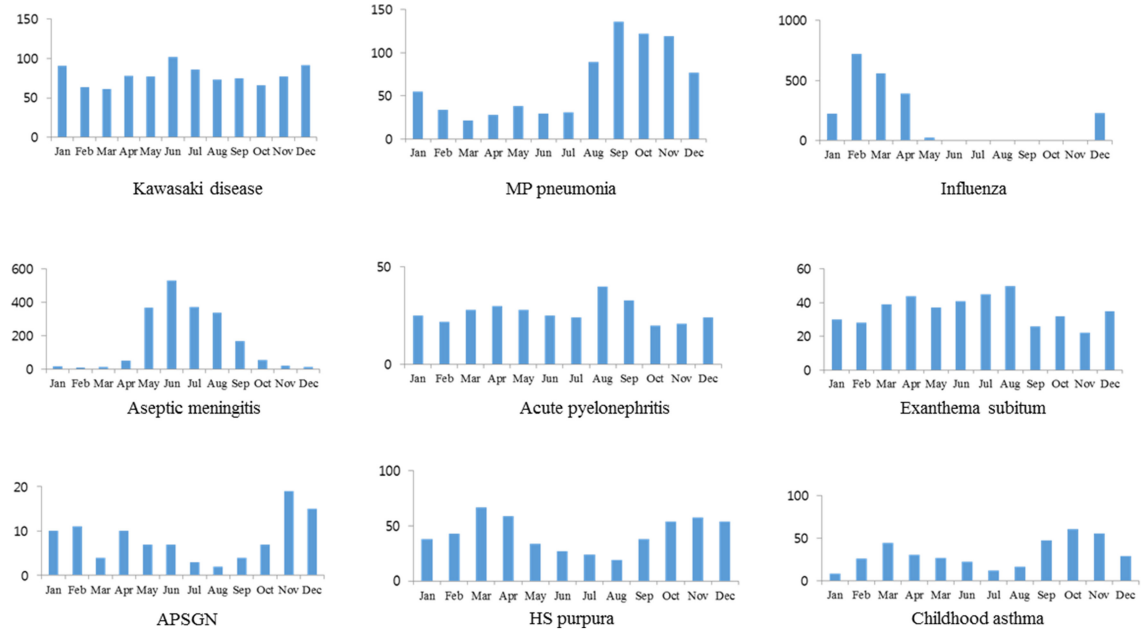

FIGURE 3 | Monthly or seasonal distribution of cases in KD and other diseases.

asthma, and APN and ES in infectious diseases. APN may be the most common systemic bacterial infection in early childhood in developed countries (23). A majority of uropathogens causing APN in infants are *E. coli* or other uropathogens such as *Klebsiella* spp., *Proteus* spp., and *Enterococcus* spp., and they may originate from the intestinal commensals in the host. It is possible that after colonization of a strain of *E. coli* or other uropathoegns transferred from other persons, the pathogens invade into the host on occasional events and elicit immune reactions (24). Although KD and APN affect mainly infants and young children, older children and adults are also affected (25). Moreover, recurrent cases are not uncommon in the both diseases. These findings suggest that immature immune function in early childhood may be associated with both diseases, and pathogens may be multiple in KD (11). ES is an acute systemic viral infection, and is characterized by sudden appearances of generalized maculopapular or morbilliform rashes just after defervescence. The etiologic agent is a species belonging to the human Herpes virus group, herpes virus type 6 or rarely type 7. Herpes viruses have a characteristic of latent infections after initial infection. ES may have no differences in age predilection, possibly sero-prevalence rate, and clinical manifestations across populations around the world and have occurred throughout every year with no seasonal variations (26, 27). Herpes virus groups become latent in the host after the initial infection as shown in herpes zoster, herpes labialis, and reactivation of cytomegalovirus and Epstein-Barr virus (EBV) in depressed immune state of the host (28). It was reported that EBV infection or herpes virus 6 infection was related to certain clinical manifestations in KD such as otorrhea or BCG inoculation site inflammation (29, 30). It is a reasonable presumption that etiologic viruses in ES may have been introduced to humans

a long-time ago, and may be coexisting within healthy human reservoirs including family members of infected infants with ES, and can be activated at any time. On occasion, the latent viruses in healthy carriers who may have a transient immune disturbance, can be reactivated and be spread to infants who have no immunity to the viruses.

In the present study, monthly case or seasonal case patterns in KD were also the most similar to those in APN or ES. Similar to this study, Nagao et al. (31) reported that the super-annual periodicity of KD was the most similar to ES among seven childhood infectious diseases including ES, herpangina, hand-foot-mouth disease, chicken pox, pharyngoconjunctival fever, erythema infectiosum, and GAS infection. They suggested that the KD pathogen is transmitted through close contact and persists asymptomatically in most hosts, likely ES (31), and this suggestion is accordance with our presumed characteristics of KD pathogens. Although the rate of KD has slowly increased with slight seasonal predominance in nationwide studies in Japan and Korea (22, 32), KD has occurred with annual fluctuations with seasonal variation each year in a given location as shown in this study. These data suggest that KD appears as local outbreaks rather than as a simultaneous nationwide epidemic as shown in viral or MP infections in Korea.

We have proposed that KD pathogens may be a species of normal flora of the host (10, 11). The disturbance of microbiota of the host, i.e., dysbiosis, has been reported in a variety of diseases, including obesity, autism spectrum disorders, allergic diseases, cancers, and autoimmune diseases such as inflammatory bowel disease, JIA and KD (33–38). It has been known that microbiota differ across ethnic groups along with different cultural environments such as diets, antibiotic use, and possibly genetic factors (39–41). Kinumaki et al. reported that *Streptococcus* spp. in intestinal microbiota markedly increased during the acute phase of KD, and suggested that KD-related streptococci may be involved in the pathogenesis of KD (38). Recently, Esposito et al. reviewed the possible role of the microbiota-host partnership on etiology and pathogenesis of KD (42). Because normal flora spread via colonization in individuals in populations, a new disease that is associated with normal flora may take a long period of time during which to spread, and would show different incidences across populations at given times as shown in KD epidemiology in Asian countries. Since pathogens exist in healthy human reservoirs as normal flora, the diseases may occur mainly in immunologically or genetically susceptible groups such as young children group that have developing immune system and microbiota (43, 44). Also, the epidemiology in these diseases can change over time along with an increasing number of people who obtain pathogens as normal flora in the populations, since normal flora that have adapted to the host may be less virulent compared to initial external pathogens. For example, scarlet fever caused by Group A beta-hemolytic streptococcus (GAS) was a severe disease in the past, with two notorious complications: acute rheumatic fever (ARF) and APSGN. Now, scarlet fever, ARF and APSGN have become rare diseases with a milder clinical phenotype in the developed countries (17, 45). However, pharyngitis caused by GAS without complications is not uncommon, and a relatively high proportion of GAS carriers among healthy children have been reported in the developed countries (46, 47). In addition, GAS strains have been susceptible to penicillin throughout the antibiotic era, with few genetic variations (48). Given that the evolutional purpose of external pathogens may be to become a species of normal flora in the host, these findings suggest that GAS strains may be changing to be part of normal flora in humans, though ARF still occurs in small populations (49). Thus, it is natural that initially severe diseases take on milder phenotypes over time, as shown in scarlet fever, pandemic influenza, and AIDS (13, 45, 50). It was also reported that recently diagnosed KD patients showed a less severe clinical phenotype, manifesting a higher incidence of incomplete KD and a lower incidence of CALs and less activated laboratory values such as C-reactive protein and albumin compared to patients who developed the disease in earlier periods (51).

Pathogenesis of KD and APN as well as other infectious and immune-mediated diseases remains to be further studied. APN has a focus in renal cortical parenchyma, where replicated uropathogens, byproducts from APN agents such as toxins and pathogen-associated molecular patterns (PAMPs), substances from injured host cells, and those from activated immune cells are produced. When these diverse substances spread from the focus into systemic circulation, clinical manifestations such as fever, tissue cell injury and/or bacteremia begin to occur, and the host immune system may respond to the substances. The majority of patients infected with APN pathogens may be asymptomatic and their disease are self-limited if systemic spread did not occur and the substances in the focus were controlled as a localized inflammation (24). On the other hand, ARF or APSGN are classic representatives of infection-related immune-mediated disease. In ARF, after 1–4 weeks after initial GAS infection when symptoms and signs of the initial bacterial infection (pharyngitis) subside, acute onset of fever and other symptoms, such as carditis, arthritis, and rarely skin rash (erythema marginatum), develop (49). The majority of patients with ARF or APSGN show a self-limited clinical course, although severely affected patients have long-term complications such as severe rheumatic heart disease or chronic renal failure, similar to giant coronary artery aneurysms in KD. Only a small proportion of patients with GAS infection are affected with ARF or APSGN. Some patients with ARF or APSGN may have preceding asymptomatic GAS infections, and other group streptococci, other bacteria or viruses are associated with postinfectious glomerulonephritis or heart tissue inflammation, including myocarditis and possibly heart valve diseases, without evidence of GAS infection (52, 53). It is believed that there are etiologic substances that induce various clinical manifestations in KD as well as in ARF and APSGN after initial infection (10). These substances include not only those originating from the pathogens but also those from the injured host cells, including damage-associated molecular patterns (DAMPs) and those from immune cells activated by infectious insults. It have been proposed that these substances bind to specific target cells of organs such as heart and joints in ARF, kidneys in APSGN, or coronary arteries or other organs in KD. The host immune system may control these substances based on their size and biochemical properties (the protein-homeostasis-system hypothesis) (10, 54, 55).

Based on the data from epidemiological and clinical studies of KD, the pathogenesis of KD could be explained as follows. Along with economic growth and westernization in East Asian countries in order of Japan, South Korea, Taiwan, and China, a species in normal flora (microbiota) in people may be affected by environmental changes such as improved hygiene or increased consumption of westernized foods including red meats (10, 11). These species (KD agents) slowly spread via colonization as normal flora to other people, including the parents and guardians of KD patients, and are eventually colonized in infants and young children predisposed to KD. On occasion, colonized KD agents invade the host via unknown events; if they are intestinal commensals, a mechanism similar to APN (via hematogeneous route in young infants) may be activated (24). Invading KD pathogens make a focus (replication site) elsewhere within the host, possibly near the upper respiratory or intestinal tracts (portal of entry) or in the target organ via systemic or local circulation. The majority of patients infected by the KD agents may be asymptomatic and have self-limited disease. However, during or after recovery from this infection, similar to ARF or APSGN, KD develops when the substances from a focus spread abruptly into systemic circulation (10). There are various incubation periods in ARF and APSGN after initial GAS infection, and no intact pathogens or structural components of the pathogen have been detected in the pathologic lesions in ARF, APSGN, KD, and other infection-related immune-mediated diseases. Furthermore, GAS strains can reside in intracellular compartments in host cells such as tonsillar epithelial cells and macrophages (56, 57), and small extracellular bacteria such as mycoplasma species can proliferate within cells and spread to other organ cells (58). Therefore, it is possible that the etiologic substances may originate from injured host cells, including a kind of DAMPs, or from immune cells exposed to infectious insults, than from various pathogens. Immune/repair system of the host, including immunoglobulins (IgG, IgM, and IgM) and platelets, begin to control the substances and take part in tissue cell repair (59). Thus, the prognoses of KD and other immune-mediated diseases depend on the ability of the host immune system to control the diverse substances from target cells injured by initial insults. Early immune modulators (corticosteroids or intravenous immunoglobulin) may act on hyperactive immune reactions performed by the non-specific adaptive immune cells (T cells and B cells) in the early stage of each disease (10, 54, 60). However, despite early treatment, a few patients with KD experience severe or giant aneurysms, and these patients may have insufficient immune function to control the substances originating from injured target cells (61). Young children with immature immune function may be prone to invasion of KD pathogens as occurs in APN in infants, and the probability of invasion of KD agents in young children may be similar across different populations (20). Since microbiota may differ across populations, marked differences in racial incidence in KD or other immune-mediated diseases such as HSP, inflammatory bowel disease, and Bechet disease may be associated with the colonization state of the different etiologic agents in the populations (20).

This retrospective study has some limitations. Epidemiological data observed in a single hospital may not match the nationwide data. However, we found that outbreaks of infectious diseases such as MP pneumonia, influenza, and aseptic meningitis in our city had occurred concurrently with nationwide epidemics. Demographic and some epidemiologic characteristics of KD, APN, APSGN, HSP, childhood asthma, and possibly ES were similar to the data in published papers in Korea (data not shown).

In conclusion, epidemiological characteristics of KD were similar to APN or ES in age distribution, annual case pattern, and seasonal variations. The pathogens of APN and ES could be regarded as one species of normal flora, since they may have originated from the host itself or ubiquitously existing human reservoirs. KD may also be associated with a species in the normal flora that may be influenced by environmental changes.

ETHICS STATEMENT

The written informed consents were obtained from the parents/caregivers of all children for the medical records to be used in this study at time of admission. The study was approved by the Institutional Review Board of The Catholic University of Korea Daejeon St. Mary's Hospital (DC18RESI0100).

AUTHOR CONTRIBUTIONS

K-YL designed the study, collected data, contributed to interpretation of results, and drafted the manuscript. J-WR contributed to data collection and drafted the initial manuscript. HK and J-WH contributed to data collection and revised the manuscript. All authors have read and approved submission of the final version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to acknowledge our colleges on data collection during the study periods of each disease.

REFERENCES

1. Kawasaki T. Acute febrile muco-cutaneous lymph node syndrome in young children with unique digital desquamation. *Arerugi.* (1967) 16:178–222.
2. Park JS, Suh CJ, Cho SH, Lee DB. Mucocutaneous lymph node syndrome: five case report. *J Korean Pediatr Soc.* (1973) 16:61–7.
3. Lue HC, Philip S, Chen MR, Wang JK, Wu MH. Surveillance of Kawasaki disease in Taiwan and review of the literature. *Acta Paediatr Taiwan.* (2004) 45:8–14.
4. Zhang T, Yanagawa H, Nakamura Y. The profiles of Kawasaki disease in China. *J Epidemiol.* (2001) 11:103–8. doi: 10.2188/jea.11.103
5. Uehara R, Belay ED. Epidemiology of Kawasaki disease in Asia, Europe, and the United States. *J Epidemiol.* (2012) 22:79–85. doi: 10.2188/jea.JE20110131

6. Singh S, Vignesh P, Burgner D. The epidemiology of Kawasaki disease: a global update. *Arch Dis Child.* (2015) 100:1084–8. doi: 10.1136/archdischild-2014-307536
7. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, treatment, and long-term management of kawasaki disease: a scientific statement for health professionals from the American Heart Association. *Circulation.* (2017) 135:e927–99. doi: 10.1161/CIR.0000000000000484
8. Kühne T. Diagnosis and management of immune thrombocytopenia in childhood. *Hamostaseologie.* (2017) 37:36–44. doi: 10.5482/HAMO-16-06-0017
9. Chen JY, Mao JH. Henoch-Schönlein purpura nephritis in children: incidence, pathogenesis and management. *World J Pediatr.* (2015) 11:29–34. doi: 10.1007/s12519-014-0534-5
10. Lee KY, Rhim JW, Kang JH. Kawasaki disease: laboratory findings and an immunopathogenesis on the premise of a "protein homeostasis system". *Yonsei Med J.* (2012) 53:262–75. doi: 10.3349/ymj.2012.53.2.262
11. Lee KY, Han JW, Lee JS. Kawasaki disease may be a hyperimmune reaction of genetically susceptible children to variants of normal environmental flora. *Med Hypotheses.* (2007) 69:642–51. doi: 10.1016/j.mehy.2006.12.051
12. Kim EK, Youn YS, Rhim JW, Shin MS, Kang JH, Lee KY. Epidemiological comparison of three *Mycoplasma pneumoniae* pneumonia epidemics in a single hospital over 10 years. *Korean J Pediatr.* (2015) 58:172–7. doi: 10.3345/kjp.2015.58.5.172
13. Rhim JW, Go EJ, Lee KY, Youn YS, Kim MS, Park SH, et al. Pandemic 2009 H1N1 virus infection in children and adults: A cohort study at a single hospital throughout the epidemic. *Int Arch Med.* (2012) 5:13. doi: 10.1186/1755-7682-5-13
14. Rhim JW, Lee KY, Youn YS, Kang JH, Kim JC. Epidemiological and clinical characteristics of childhood pandemic 2009 H1N1 virus infection: an observational cohort study. *BMC Infect Dis.* (2011) 11:225. doi: 10.1186/1471-2334-11-225
15. Lee KY, Burgner D, Lee HS, Hong JH, Lee MH, Kang JH, et al. The changing epidemiology of pediatric aseptic meningitis in Daejeon, Korea from 1987 to 2003. *BMC Infect Dis.* (2005) 5:97. doi: 10.1186/1471-2334-5-97
16. Huh SM, Park BK, Kang HM, Rhim JW, Suh JS, Lee KY. Clinical implications of DMSA scan in childhood acute pyelonephritis. *Child Kidney Dis.* (2017) 21:107–13. doi: 10.3339/jkspn.2017.21.2.107
17. Kuem SW, Hur SM, Youn YS, Rhim JW, Suh JS, Lee KY. Changes in acute poststreptococcal glomerulonephritis: an observation study at a single Korean hospital over two decades. *Child Kidney Dis.* (2015) 19:112–7. doi: 10.3339/chikd.2015.19.2.112
18. Rhim JW, Kang HM, Yang EA, KY Lee. Epidemiological relationship between *Mycoplasma pneumoniae* pneumonia and recurrent wheezing episode in children: an observational study at a single hospital in Korea. *BMJ Open.* (2019) 9:e026461. doi: 10.1136/bmjopen-2018-026461
19. Lee KY, Han JW, Lee HS, Hong JH, Hahn SH, Lee JS, et al. Epidemiologic study of Kawasaki disease at a single hospital in Daejeon, Korea (1987 through 2000). *Pediatr Infect Dis J.* (2004) 23:52–5. doi: 10.1097/01.inf.0000105201.92839.ec
20. Rhim JW, Youn YS, Han JW, Lee SJ, Oh JH, Lee KY. Changes in Kawasaki disease during 2 decades at a single institution in Daejeon, Korea. *Pediatr Infect Dis J.* (2014) 33:372–5. doi: 10.1097/INF.0000000000000123
21. Park YW, Park IS, Kim CH, Ma JS, Lee SB, Kim CH, et al. Epidemiologic study of Kawasaki disease in Korea, 1997-1999: comparison with previous studies during 1991-1996. *J Korean Med Sci.* (2002) 17:453–6. doi: 10.3346/jkms.2002.17.4.453
22. Kim GB, Park S, Eun LY, Han JW, Lee SY, Yoon KL, et al. Epidemiology and clinical features of Kawasaki disease in South Korea, 2012-2014. *Pediatr Infect Dis J.* (2017) 36:482–5. doi: 10.1097/INF.0000000000001474
23. Bhat RG, Katy TA, Place FC. Pediatric urinary tract infections. *Emerg Med Clin North Am.* (2011) 29:637–53. doi: 10.1016/j.emc.2011.04.004
24. Lee KY. New insights for febrile urinary tract infection (acute pyelonephritis) in children. *Child Kidney Dis.* (2016) 20:37–44. doi: 10.3339/jkspn.2016.20.2.37
25. Kontopoulou T, Kontopoulos DG, Vaidakis E, Mousoulis GP. Adult Kawasaki disease in a European patient: a case report and review of the literature. *J Med Case Rep.* (2015) 9:75. doi: 10.1186/s13256-015-0516-9
26. Enders G, Biber M, Meyer G, Helftenbein E. Prevalence of antibodies to human herpesvirus 6 in different age groups, in children with exanthema subitum, other acute exanthematous childhood diseases, Kawasaki syndrome, and acute infections with other herpesviruses and HIV. *Infection.* (1990) 18:12–5. doi: 10.1007/BF01644173
27. Agut H, Bonnafous P, Gautheret-Dejean A. Update on infections with human herpesviruses 6A, 6B, and 7. *Med Mal Infect.* (2017) 47:83–91. doi: 10.1016/j.medmal.2016.09.004
28. Collins-McMillen D, Goodrum FD. The loss of binary: Pushing the herpesvirus latency paradigm. *Curr Clin Microbiol Rep.* (2017) 4:124–31. doi: 10.1007/s40588-017-0072-8
29. Pavone P, Cocuzza S, Passaniti E, Longo MR, Verrotti A, Serra A, et al. Otorrhea in Kawasaki disease diagnosis complicated by an EBV infection: coincidental disease or a true association. *Eur Rev Med Pharmacol Sci.* (2013) 17:989–93.
30. Kakisaka Y, Ohara T, Katayama S, Suzuki T, Sasai S, Hino-Fukuyo N, et al. Human herpes virus type 6 can cause skin lesions at the BCG inoculation site similar to Kawasaki disease. *Tohoku J Exp Med.* (2012) 228:351–3. doi: 10.1620/tjem.228.351
31. Nagao Y, Urabe C, Nakamura H, Hatano N. Predicting the characteristics of the aetiological agent for Kawasaki disease from other paediatric infectious diseases in Japan. *Epidemiol Infect.* (2016) 144:478–92. doi: 10.1017/S0950268815001223
32. Makino N, Nakamura Y, Yashiro M, Ae R, Tsuboi S, Aoyama Y, et al. Descriptive epidemiology of Kawasaki disease in Japan, 2011-2012: from the results of the 22nd nationwide survey. *J Epidemiol.* (2015) 25:239–45. doi: 10.2188/jea.JE20140089
33. Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Curr Opin Pharmacol.* (2009) 9:737–43. doi: 10.1016/j.coph.2009.06.016
34. Doenyas C. Gut microbiota, inflammation, and probiotics on neural development in autism spectrum disorder. *Neuroscience.* (2018) 374:271–86. doi: 10.1016/j.neuroscience.2018.01.060
35. Hörmannsperger G, Clave T, Haller D. Gut matters: microbe-host interactions in allergic diseases. *J Allergy Clin Immunol.* (2012) 129:1452–9. doi: 10.1016/j.jaci.2011.12.993
36. Chen J, Domingue JC, Sears CL. Microbiota dysbiosis in select human cancers: evidence of association and causality. *Semin Immunol.* (2017) 32:25–34. doi: 10.1016/j.smim.2017.08.001
37. Arvonen M, Berntson L, Pokka T, Karttunen TJ, Vähäsalo P, Stoll ML. Gut microbiota-host interactions and juvenile idiopathic arthritis. *Pediatr Rheumatol Online J.* (2016) 14:44. doi: 10.1186/s12969-016-0104-6
38. Kinumaki A, Sekizuka T, Hamada H, Kato K, Yamashita A, Kuroda M. Characterization of the gut microbiota of Kawasaki disease patients by metagenomic analysis. *Front Microbiol.* (2015) 6:824. doi: 10.3389/fmicb.2015.00824
39. Lin L, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunol.* (2017) 18:2. doi: 10.1186/s12865-016-0187-3
40. Harusato A, Chassaing B. Insights on the impact of diet-mediated microbiota alterations on immunity and diseases. *Am J Transplant.* (2018)18:550–5. doi: 10.1111/ajt.14477
41. Korpela K, de Vos WM. Antibiotic use in childhood alters the gut microbiota and predisposes to overweight. *Microb Cell.* (2016) 3:296–8. doi: 10.15698/mic2016.07.514
42. Esposito S, Polinori I, Rigante D. The gut microbiota-host partnership as a potential driver of Kawasaki syndrome. *Front Pediatr.* (2019) 7:124. doi: 10.3389/fped.2019.00124
43. Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. *Pediatr Allergy Immunol.* (2014) 25:428–38. doi: 10.1111/pai.12232
44. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci.* (2015) 282:20143085. doi: 10.1098/rspb.2014.3085
45. Quinn RW. Comprehensive review of morbidity and mortality trends for rheumatic fever, streptococcal disease, and scarlet fever: the decline of rheumatic fever. *Rev Infect Dis.* (1989) 11:928–53.

46. Kaplan EL, Gastanaduy AS, Huwe BB. The role of the carrier in treatment failures after antibiotic for group A streptococci in the upper respiratory tract. *J Lab Clin Med.* (1981) 98:326–35.
47. Roberts AL, Connolly KL, Kirse DJ, Evans AK, Poehling KA, Peters TR, et al. Detection of group A Streptococcus in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage. *BMC Pediatr.* (2012) 12:3. doi: 10.1186/1471-2431-12-3
48. Brook I. Treatment challenges of Group A beta-hemolytic streptococcal pharyngo-tonsillitis. *Int Arch Otorhinolaryngol.* (2017) 21:286–96. doi: 10.1055/s-0036-1584294
49. Gewitz MH, Baltimore RS, Tani LY, Sable CA, Shulman ST, Carapetis J, et al. Revision of the Jones Criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography: a scientific statement from the American Heart Association. *Circulation.* (2015) 131:1806–18. doi: 10.1161/CIR.0000000000000205
50. Mirani G, Williams PL, Chernoff M, Abzug MJ, Levin MJ, Seage GR III, et al. IMPAACT P1074 Study Team. Changing trends in complications and mortality rates among US youth and young adults with HIV Infection in the era of combination antiretroviral therapy. *Clin Infect Dis.* (2015) 61:1850–61. doi: 10.1093/cid/civ687
51. Kil HR, Yu JW, Lee SC, Rhim JW, Lee KY. Changes in clinical and laboratory features of Kawasaki disease noted over time in Daejeon, Korea. *Pediatr Rheumatol.* (2017) 15:60. doi: 10.1186/s12969-017-0192-y
52. Kambham N. Postinfectious glomerulonephritis. *Adv Anat Pathol.* (2012) 19:338–47. doi: 10.1097/PAP.0b013e31826663d9
53. Trachtenberg BH, Hare JM. Inflammatory cardiomyopathic syndromes. *Circ Res.* (2017) 121:803–18. doi: 10.1161/CIRCRESAHA.117.310221
54. Lee KY. A common immunopathogenesis mechanism for infectious diseases: the protein-homeostasis-system hypothesis. *Infect Chemother.* (2015) 47:12–26. doi: 10.3947/ic.2015.47.1.12
55. Lee KY. A unified pathogenesis for kidney diseases, including genetic diseases and cancers, by the protein-homeostasis-system hypothesis. *Kidney Res Clin Pract*. (2017) 36:132–44. doi: 10.23876/j.krcp.2017.36.2.132
56. Osterlund A, Engstrand L. An intracellular sanctuary for *Streptococcus pyogenes.* in human tonsillar epithelium: studies of asymptomatic carriers and *in vitro* cultured biopsies. *Acta Otolaryngol.* (1997) 117:883–8.
57. O'Neill AM, Thurston TL, Holden DW. Cytosolic replication of *Group A streptococcus.* in human macrophages. *MBio.* (2016) 7:e00020–16. doi: 10.1128/mBio.00020-16
58. Hegde S, Hegde S, Spergser J, Brunthaler R, Rosengarten R, Chopra-Dewasthaly R. *In vitro* and *in vivo* cell invasion and systemic spreading of *Mycoplasma agalactiae* in the sheep infection model. *Int J Med Microbiol.* (2014) 304:1024–31. doi: 10.1016/j.ijmm.2014.07.011
59. Han JW, Oh JH, Rhim JW, Lee KY. Correlation between elevated platelet count and immunoglobulin levels in the early convalescent stage of Kawasaki disease. *Medicine (Baltimore).* (2017) 96:e7583. doi: 10.1097/MD.0000000000007583
60. Lee KY. Pneumonia, acute respiratory distress syndrome, and early immune-modulator therapy. *Int J Mol Sci.* (2017) 18:E388. doi: 10.3390/ijms 18020388
61. Seo YM, Kang HM, Lee SC, Yu JW, Kil HR, Rhim JE, et al. Clinical implications in laboratory parameter values in acute Kawasaki disease for early diagnosis and proper treatment. *Korean J Pediatr.* (2018) 61:160–6. doi: 10.3345/kjp.2018.61.5.160

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

frontiers in Pediatrics

MINI REVIEW
published: 18 June 2019
doi: 10.3389/fped.2019.00242

Biomarkers for Kawasaki Disease: Clinical Utility and the Challenges Ahead

*Himanshi Chaudhary, Johnson Nameirakpam, Rajni Kumrah, Vignesh Pandiarajan, Deepti Suri, Amit Rawat and Surjit Singh**

Post Graduate Institute of Medical Education and Research, Chandigarh, India

Kawasaki disease (KD) has replaced acute rheumatic fever as the most common cause of acquired heart disease in children in the developed world and is increasingly being recognized from several developing countries. It is a systemic vasculitis with a predilection for coronary arteries. The diagnosis is based on a constellation of clinical findings that appear in a temporal sequence. Quite understandably, this can become a problem in situations wherein the clinical features are not typical. In such situations, it can be very difficult, if not impossible, to arrive at a diagnosis. Several biomarkers have been recognized in children with acute KD but none of these has reasonably high sensitivity and specificity in predicting the course of the illness. A line up of inflammatory, proteomic, gene expression and micro-RNA based biomarkers has been studied in association with KD. The commonly used inflammatory markers e.g. erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and total leucocyte counts (TLC) lack specificity for KD. Proteomic studies are based on the identification of specific proteins in serum, plasma and urine by gel electrophoresis. A host of genetic studies have identified genes associated with KD and some of these genes can predict the course and coronary outcomes in the affected individuals. Most of these tests are in the early stages of their development and some of these can predict the course, propensity to develop coronary artery sequelae, intravenous immunoglobulin (IVIg) resistance and the severity of the illness in a patient. Development of clinical criteria based on these tests will improve our diagnostic acumen and aid in early identification and prevention of cardiovascular complications.

Keywords: immunology, vasculitis, Kawasaki, biomarkers, childhood vasculitis

OPEN ACCESS

Edited by:
Kyung-Yil Lee,
The Catholic University of Korea,
South Korea

Reviewed by:
Diego F. Coutinho,
New York University, United States
Hongryang Kil,
Chungnam National University,
South Korea

****Correspondence:***
Surjit Singh
surjitsinghpgi@rediffmail.com

Specialty section:
This article was submitted to Pediatric Immunology, a section of the journal Frontiers in Pediatrics

Received: *16 January 2019*
Accepted: *28 May 2019*
Published: *18 June 2019*

Citation:
Chaudhary H, Nameirakpam J, Kumrah R, Pandiarajan V, Suri D, Rawat A and Singh S (2019) Biomarkers for Kawasaki Disease: Clinical Utility and the Challenges Ahead. Front. Pediatr. 7:242. doi: 10.3389/fped.2019.00242

INTRODUCTION

Kawasaki disease (KD) is a common childhood vasculitis. The disease was first described in Japanese children in 1967 by a Japanese pediatrician, Dr. Tomisaku Kawasaki (1). The highest incidence is seen in Japan, Korea and Taiwan (2). However, the disease is now being reported world over including several developing countries like India (3–7).

The diagnosis of KD is essentially clinical (8). The clinical features mimic many self-resolving exanthematous febrile illnesses of childhood (e.g., measles, adenoviral infection, scarlet fever, dengue fever). These illnesses share some common clinical features with KD like fever, rash, mucocutaneous manifestations, lymphadenopathy, and elevated inflammatory parameters. The diagnosis of KD can be easily missed if the cascade of clinical findings goes unrecognized especially in cases of incomplete KD. Things can get even more complicated when KD occurs in association with an infection. The associated coronary artery abnormalities (CAAs) may go undetected and can have significant long-term implications if treatment is not initiated at the right time. Unlike other vasculitides and other rheumatological disorders, there are no pathognomonic laboratory tests for diagnosis of this condition. For the treating clinician, it is often difficult to confirm a diagnosis of KD on the bedside. It is, therefore, important to establish a set of laboratory markers that are sensitive, specific, reproducible and help the treating pediatrician in arriving at a diagnosis. As intravenous immunoglobulin (IVIg) is an expensive product, it is imperative that it be used only in situations where it is definitely indicated. However, in a setting of incomplete KD, the pediatrician often faces the dilemma of under-treatment with its attendant risks of CAAs vs. using IVIg in circumstances where it may not really be indicated.

Several biomarkers have been studied in association with KD and some of these have been shown to be predictive of resistance to IVIg while others may be indicative of an increased risk of development of CAAs. This manuscript overviews some of the important biomarkers that have been studied in KD and highlights their role in the diagnosis and assessment of disease severity.

INFLAMMATORY BIOMARKERS (TABLE 1)

These are the conventional biomarkers that mirror inflammatory activity and are not specific to KD. Erythrocyte sedimentation rate (ESR) is consistently elevated during the acute phase of KD but may be unreliable as a marker of disease activity after the administration of IVIG (9–11). There is neutrophilic leukocytosis during the acute phase of the disease and the degree of leukocytosis has been correlated with myocardial dysfunction (10, 12). Thrombocytosis, usually seen after the completion of the first week of illness, is a marker of ongoing inflammation. Persistent thrombocytosis has been linked to the development of CAAs but the association is tenuous (9, 18). C-reactive protein (CRP) is known to have a significant association with disease severity and the development of CAAs (9, 10, 12). Procalcitonin levels have been shown to be elevated during the acute phase and this rise is especially marked in children with resistance to IVIg (13, 16). Peripheral blood eosinophilia (PBE) (17) and low albumin has been associated with increased risk of IVIG resistance and coronary complications (14). Low mean platelet volume (MPV) (15), platelet distribution width (PDW) (19), and platelet-derived microparticles (PDMP) (20) have been shown to be markers of platelet activation and inflammation in acute stages of KD. However, their use as biomarkers for the disease requires replication of results across different populations.

Summary

Inflammatory markers are largely nonspecific as these are also elevated in many other inflammatory and infective conditions. In a clinical setting of KD, while these biomarkers can reflect ongoing inflammation, they are of limited use in arriving at a definitive diagnosis.

IMMUNOLOGICAL MARKERS (TABLE 2)

Cellular Markers

Innate Immunity

Myeloid dendritic cells (mDCs), which serve as a bridge between adaptive and innate immunity, have been shown to be decreased in acute KD. Takahashi et al. (21) have suggested that decreased levels are due to either recirculation into affected tissues or

TABLE 1 | Inflammatory biomarkers of Kawasaki disease.

Parameter	Normal values	Comment	References
Erythrocyte sedimentation rate (ESR)	0–22 mm/h	• Increased in acute phases • Unreliable for monitoring response to IVIG therapy	(6–8)
Total leucocyte count(TLC)	4–11 × 109/L	• Higher counts associated with higher risk of CAAs • High in patients with delayed diagnoses of KD	(9–12)
Platelet count	150–400 × 109/L	Increased in acute stage and prolonged thrombocytosis associated with increased risk of CAAs	(6, 13)
Mean platelet volume(MPV)	7–11 fl	Low values increase the likelihood of CAAs	(14)
Platelet distribution width(PDW)	10.0–17.9%	High values suggest platelet activation and increase the likelihood of CAAs	(15)
C-Reactive protein(CRP)	<10 mg/L	Prediction of cardiac sequelae, age-dependent prognosis	(10, 11, 13)
Procalcitonin	<0.15 ng/mL	Increased in acute stage; will help differentiate acute KD from viral infections	(16, 17)
Peripheral blood eosinophilia (PBE)	0.0–6.0%	Higher rates in acute stages of incomplete KD; may be helpful in clinical setting of incomplete KD	(18)

TABLE 2 | Immunological biomarkers of Kawasaki disease.

Cellular markers	Biological functions	Comments	References
CD8 T cells	Cytotoxic T cell	Decrease in acute KD; shown to sequester in inflamed coronary arteries, functionally suppressed	(21–23)
Th1 cells	Regulate cellular immunity by secreting IL-2 and IFN-γ	Downregulated in acute KD	(24)
Th2 cells	Regulate humoral immunity by secreting IL-4, IL-5, IL-6 and IL-10	Downregulated in acute KD and involved in response to IVIG	(21, 24)
CD14+ monocytes	Produce TNFα, IL-6, IL-1	Increased in acute stages and in association with CAAs	(21)
CD69+CD8T cells	Early activation marker for T cells	Increased in acute KD; marker to determine disease progression, treatment response, and convalescence in acute KD	(25)
Effector memory T-cells (Tem)	Found in the peripheral circulation and tissues; provide the immune system with "memory" against previously encountered pathogens.	Increase after IVIG treatment	(25)
Regulatory T cells (Treg)	Maintain tolerance to self-antigens	Decreased in acute KD, Increase after IVIG treatment	(25, 26)
Central memory T-cells (Tcm)	Found in the lymph nodes and in the peripheral circulation, provide the immune system with "memory" against previously encountered pathogens.	Increase in acute KD	(25)
Myeloid and plasmocytoid dendritic cells (DC	Most potent antigen presenting cells that initiates T-cell activation.	mDC increase in acute KD No increase in pDC	(20)
Th17 proportions	Regulate inflammation by secreting IL-17	Decreased in acute KD, Increase after IVIG treatment	(27)
IFN-Y and IL-2	Th1 cytokine	Elevated in acute KD	(24)
IL-4, IL-10	Th2 cytokine	Elevated in acute KD	(24)
IL-6	Important mediator of the acute phase response	Upregulated in acute stages, more elevated in IVIG refractory cases	(24)
IL-17A/F, ROR-gt	Induce IL-6 production	Upregulated in acute stages; responsible for signs of inflammation	(28)
TGF-b	Marker of macrophage activation	Higher in acute stages, associated with CAAs	(29)
TNFa	Mediate endothelial cell activation	Increase in acute KD, role in CAAs	(30)
CXCL10 (IP-10)	Th1 associated chemokine	Upregulated in acute KD	(31, 32)
CCL-2	Th2 associated chemokine	Activation in acute KD	(31, 32)

increased peripheral destruction in cases of acute KD. Furukawa et al. (22) studied CD14+ macrophages in KD patients and showed that they were higher in those with CAAs, thereby suggesting that absolute counts of CD14+ monocytes can be a marker of the severity of KD. These studies point toward a possible dysfunction in the innate immunity axis which could contribute to the inflammatory upregulation seen in acute stages of KD.

Acquired Immunity

Studies have shown a decreased number of CD8 T cells during acute stages of KD (23). Immunohistochemistry studies in coronary arteries of KD patients at autopsy have shown that CD8 T cells preferentially sequester in the coronary arteries and are responsible for the inflammatory vasculitis (24, 26). Ehara et al. (27) showed that markers of early T cell activation [CD69(+)CD8T cells] increased in acute stages and can be used as a marker of disease progression and response to IVIg. Helper T cells (Th1 and Th2) have been shown to be upregulated during acute stages (22, 25). There is an apparent imbalance of T helper 17 cells (Th17) and regulatory T cells (Tregs) in acute KD. Th17 proportions have been shown to be upregulated while Treg proportions were found to be downregulated during the acute phase of KD (33).

Soluble Markers

Soluble markers of inflammation have been studied at great length in association with KD. Plasma levels of Th1 (IFN-Y, IL-12) and Th2 (IL-4, IL-13) cytokines have been shown to be elevated during acute stages of KD (34). Multiple pro-inflammatory and anti-inflammatory cytokines have been studied in acute stages of KD, but none of these has been standardized as a biomarker of KD (29). Zhou et al. (28) showed a close relation between levels of vascular endothelial growth factor (VEGF), IL-6, and development of CAAs. TGF-β signaling has been implicated in the development of coronary artery aneurysms (29). Th1-associated (CXCL10) and Th2-associated chemokines (CCL2) are elevated in acute stages of KD and have been shown to decrease with IVIG (35, 36). Tumor Necrosis Factor α (TNFα) has a role in the recruitment of inflammatory cells to coronary endothelium and has been shown to have a role in the development of CAAs. TNFα blocking agents have been extensively studied as a therapy for KD (37).

Summary

Data on immunological markers in KD are derived from studies on relatively small groups of patients. The significance of these biomarkers in predicting the disease course, response to therapy

and complications is not clear and needs confirmation across a different population.

Proteomic Biomarkers (Table 3)

Several proteins have been studied in KD.

N-terminal Prohormone of Brain Natriuretic Peptide (NT-proBNP)

NT-proBNP has been used as a marker of myocardial damage in KD. It is a marker of cardiomyocyte stress imposed by pressure or volume overload and is increased in response to cardiac dilatation and neuro-humoral factors. According to Dahdah et al.

TABLE 3 | Proteomic biomarkers of Kawasaki disease.

Protein	Biological function	Caveats	Limitations	References
NT-pro BNP	Marker of myocardial damage; increased in response to cardiac dilatation and neuro-humoral factors	Higher values in CAAs and can predict IVIG resistance	Non-specific test • Can be elevated in other causes of diastolic dysfunction • Serum values vary with age	(38–44)
Suppression of tumorigenicity 2(sST2)	Member of the IL 1 receptor family and reflect cardiovascular stress and fibrosis	Elevated in acute stages of KD Correlate with impaired myocardial relaxation	Prognostic significance of sST2 levels in acute KD is unknown	(45)
Cardiac troponin I (cTnI)r	Marker of myocardial damage	Elevated in acute stages	Non-specific marker	(46, 47)
Periostin	Matricellular protein that plays a role in vascular and cardiac responses to injury	Upregulated 11-fold in acute and chronic KD coronary arteries	Non-specific	(48)
Gamma-glutamyl transferase(GGT) and Alanine transferase (ALT)	Biomarkers of cardiocyte inflammation	Increased in acute stages of KD	Non-specific	(45)
Clusterin	Component of high density lipoproteins; role in maintaining integrity of coronary endothelium	Values < 12 mg/L associated with CAAs occurrence in KD patients	Need validation via larger studies	(49)
Thrombospondin (TSP-1 and TSP-2)	Involved in cardiovascular inflammation and maintaining the integrity and function of cardiac structures	• Elevated in acute KD • Associated with high risk of IVIg resistance	Need larger studies for validation	(50)
Fibrinogen beta and gamma chains	Cleavage products of fibrinogen and fibrin regulate systemic inflammation	Elevated in acute KD	Non-specific markers of inflammation	(49)
CD5 antigen-like precursor (CD5L)	Marker of acute inflammation	Increased in acute KD	Non-specific markers of inflammation	(49)
Nitric oxide synthases (iNOS)	NO has an important role in maintaining vascular tone and integrity of vessels	Correlate with the severity and progression of CAA	Non-specific marker of inflammation	(51)
Periostin	Matricellular protein of coronary endothelium	KD patients have significantly elevated serum values compared with febrile controls	Tissue based tests are difficult in clinical settings	(44)
Lipopolysaccharide-binding protein (LBP)	Markers of inflammation	Higher in acute KD	Need validation in larger studies	(52)
Leucine-rich alpha-2-glycoprotein (LRG1)	Markers of inflammation	Higher in acute KD	Need validation in larger studies	(52)
Angiotensinogen (AGT)	Markers of inflammation	Higher in acute KD	Need validation in larger studies	(52)
Tenacin- C	Extracellular matrix glycoprotein that is upregulated at sites of tissue injury and inflammation	Useful biomarker to predict the risk of developing CAAs and IVIg resistance	Need validation in larger studies	(53)
Urine protein markers: • Filamin • Talin • Complement regulator CSMD3 • Immune pattern recognition receptor muclin • Immune cytokine protease meprin A	Markers of inflammation	• Higher in acute KD • Non-invasive biomarkers of KD	Need validations via larger studies	(54)

(31), myocardial involvement in acute KD is universal from the histological and functional perspectives and hence the role of NT-proBNP as a potential biomarker has been extensively studied. The interpretation of serum NT-proBNP levels in children is difficult because these are age-dependent, being highest in infancy and decreasing thereafter. The sensitivity and specificity of cut-off values as a biomarker for KD are derived from receiver operating characteristics (ROC) analysis, the upper normal limit for age, and Z-scores for age. Shiraishi et al. (32) showed that the sensitivity and specificity of diagnosing acute KD were 97.8 and 47%, respectively, at a z score > 2. A meta-analysis on NT-proBNP in KD has substantiated its use as a diagnostic marker and cut-off values between 96 and 260 pg/ml have been shown to have sensitivity between 66 and 98% in identifying cases of KD (30). NT-proBNP levels are higher in patients with CAAs (values 515–1,300 pg/ml have a sensitivity and specificity of 73–95 and 61–85%, respectively) (38) and can predict IVIG resistance. A value between 629 and 1,300 pg/ml has a high sensitivity (70–79%) as well as specificity (58–77%) for diagnosis of KD (39, 40). From our center, we have reported a cut-off at 1,025 pg/mL for NT-proBNP levels which has a sensitivity of 88% and specificity of 96% (41) in the acute phase of KD.

Other Cardiovascular Biomarkers

Suppression of tumorigenicity-2 (sST2) is a member of the interleukin 1 (IL-1) receptor family and reflects cardiovascular stress and fibrosis. It is elevated in acute stages of KD and the levels correlate with impaired myocardial relaxation. However, the prognostic significance in acute KD is unclear (42).

Kim et al. (43) have described cardiac troponin I (cTnI) in relation with KD and showed a significant increase in the level of cTnI in the acute stage of KD. Periostin is a matricellular protein that mediates responses associated with cardiovascular injury. Reindel et al. (44) have shown that periostin gets upregulated in coronary arteries during the acute and chronic phases of KD when compared to other febrile controls. Gamma-glutamyl transferase (GGT) and alanine transferase (ALT) have also been studied as biomarkers of cardiac inflammation. However, these are rather nonspecific and are not a reliable marker for KD *per se* (42).

Thrombospondin (TSP-1 and TSP-2)

TSP-1 and TSP-2 are proteins involved in cardiovascular inflammation and maintaining the integrity and function of cardiac structures. These have been shown to be elevated in the acute phase of KD in comparison with other febrile controls and higher values are seen in those with IVIg resistance. With a cut-off value of 31.50 ng/mL, the sensitivity of TSP-2 has been shown to be 82.35% and specificity 64.81% in predicting IVIG resistance in acute KD (45).

Other Proteins

Clusterin is a part of the high-density lipoproteins (HDL) and has a role in many physiologic processes including maintaining the integrity of coronary artery walls. Plasma clusterin levels have been studied in KD and values lower than 12 mg/L have been associated with the occurrence of CAAs in KD patients (46). Yu et al. (46, 55) have studied several proteins of fibrinogen cascade in KD. It was found that these were increased in patients with KD and may serve as a good biomarker of KD. The expression of nitric oxide synthases (iNOS) has been shown to correlate with the extent of coronary damage and progression of CAAs in patients with acute KD (47). Tenascin- C (TN-C) is a marker of tissue injury and inflammation and has a role in the maintenance of the extracellular matrix of cardiac tissue. Okuma et al. (48) have studied serum TN-C level in association with the risk of developing CAAs and resistance to IVIg therapy in the acute phase of KD.

Summary

Several protein biomarkers have been studied in relation to KD but most of these studies are based in small cohorts of patients at a single center. NT-proBNP is widely believed to be a useful marker for confirmation of the diagnosis of KD at the bedside. The levels of NT-proBNP in KD, however, may overlap those in other febrile illnesses with cardiac dysfunction. Other biomarkers are still in their early stages of discovery and will require validation in larger populations before the results can be used in clinical settings.

Urine Biomarkers

Kentsis et al. (49) studied urine protein biomarkers in relation with KD and showed an abundance of markers of tissue injury (filamin and talin), complement regulator (CSMD3), cytokine protease (meprin A), and immune pattern recognition receptor (muclin) in the urine of affected patients. However, these results need replication in larger studies before these can be used as noninvasive biomarkers in KD.

GENETIC STUDIES IN KD (TABLE 4)

A genetic basis of KD has been strongly considered taking into account the geographical differences in the incidence and high risk of occurrence in family members. Incidence of KD in Japan, Korea and Taiwan is more than 10 times the incidence in Western countries (50–53). This difference is a pointer toward a possible genetic susceptibility or may reflect environmental or lifestyle differences amongst these populations. A higher incidence is also known amongst Japanese Americans settled in Hawaii and this is comparable with the incidence in Japan, further pointing toward a possible genetic association of the disease (75). Studies have shown that the risk of developing KD in siblings of a KD patient is 10–30-fold higher as compared to the general population (76). Two types of approaches have been utilized for studying the genetic basis of KD:

1. Candidate gene approach
2. Genome-wide approach

Candidate Gene Approach

Candidate gene studies are the well-known approach to study genes associated with KD based on their function in the pathophysiology of the disease.

TABLE 4 | Genetic biomarkers of Kawasaki disease.

Gene	Biological function	Study	Year and country	Ethnicity	Polymorphism	Conclusion	References
ITPKC	Calcium channel modulator and regulates calcium release from ER, Acts as negative regulator of T cell activation	Wang et al.	2014, China	Asian	rs2720378, rs2069762	Higher risk of developing KD	(56)
		Peng et al.	2012, China	Asian	rs2290692	Higher risk of developing KD	(57)
		Kou et al.	2011, Taiwan	Asian	rs28493229	Higher risk of developing KD and higher risk of CAAs	(58)
ORAI1	Involved in calcium influx into T-cells and activation of the Ca2+/NFAT pathway, regulates immune system and inflammatory responses	Onouchi et al.	2016, Japan	Asian	rs3741596	Higher risk of developing KD	(59)
CD40	Activates immune system and is involved in immune and inflammatory responses	Lou et al.	2016, Japan	Asian	rs2736340, rs4813003, rs3818298	Higher risk of developing KD	(60)
		Cheng et al.	2015, China	Asian	rs1801274	Higher risk of developing KD	(61)
		Onouchi et al.	2012, Japan	Asian	rs1535045, rs4813003	Higher risk of developing KD	(62)
BLK	Involved in signal transduction and phosphorylation of ITAM residues of Iga and Igb, Responsible for B cell activation	Lou et al.	2015, China	Asian	rs2736340	Higher risk of developing KD	(60)
		Chang et al.	2013, Taiwan	Asian	rs2736340	Higher risk of developing KD	(63)
		Lee et al.	2012, Taiwan	Asian	rs2618476, rs2736340	Higher risk of developing KD	(64)
FCGR2A	Involved in metabolism and turnover of circulating IgG, Required for phagocytosis and clearing of immune complexes	Duan et al.	2014, China	Asian	rs1801274	Higher risk of developing KD	(65)
		Khor et al.	2013, Singapore	Mixed	rs1801274	Higher risk of developing KD	(66)
		Yan et al.	2013, China	Asian	rs1801274	Higher risk of developing CAAs in KD	(67)
CASP3	Involved in cell apoptosis, regulates cellular processes in T cells	Wang et al.	2014, China	Asian	rs2069762, rs2720378	Higher risk of developing KD	(68)
		Onouchi et al.	2010, Japan	Asian	rs72689236	Higher risk of developing KD	(69)
		Kou et al.	2011, Taiwan	Asian	rs72689236	Higher risk of developing KD	(70)
TGFβR2	Regulation of gene transcription	Choi et al.	2012, Korea	Asian		Higher risk of developing KD	(71)
SMAD3	Signal transducer and transcriptional modulator, involved in down-regulation of T-cells and cardiovascular remodeling	Kuo et al.	2011, Taiwan	Asian	rs1438386	Higher risk of developing KD, but not to CAAs	(72)
		Peng et al.	2016, China	Asian	rs1438386	Higher risk of developing KD	(73)
ADAM17	Required for activation of notch signaling pathway and processing	Peng et al.	2016, China	Asian	rs6705408	Higher risk of developing KD and development of CAAs	(73)
MMP-11	Causes breakdown of extracellular matrix	Ban et al.	2010 Korea	Asian	rs738792	Higher risk of developing KD	(74)

HLA Genes

Early genetic studies on KD were focused on HLA alleles. HLA class 1 antigens have been studied in great detail in various populations (54, 77). Significant predominant association of HLA-Bw54 has been found in Japanese KD population while single nucleotide polymorphisms (SNP) located in the *HLA-E* gene was suggested to be associated with KD in the Han Chinese population (77). A positive association was found between HLA-Bw51 and KD in the White and Jewish population while HLA-Bw51 was reported to be negatively associated in the Korean population. In a recent GWAS, association with HLA class II antigens peaked at the intergenic region between HLA-DQB2 and HLA-DOB (78). SNPs in HLA class III region have also been described in association with KD (79). TNF∞ expression has been shown to be elevated in association with CAAs in KD in Korean, Taiwanese, Chinese and Caucasian populations, but these results could not be replicated in studies from Japan (80).

Non-HLA Genes

Burns et al. (78) found an SNP in the promoter region of the IL-4 gene to be asymmetrically transmitted in children with KD. MHC-class-I-chain-related gene A (MICA) alleles were reported as biomarkers for evaluation of coronary aneurysms in KD.

Lower frequency of MICA allele 276 A4 was reported in KD patients with aneurysms (81).

Genome-Wide Approach

Genome-Wide Linkage Analyses (GWLS)

GWLS were used to map the genetic loci of diseases by analyses of related individuals through the logarithm of odds (LOD) score. The first GWLS on KD was done by Onouchi et al. (82) who studied 79 families of children with KD and analyzed 75 sibling pairs. They identified 10 chromosomal loci with positive linkage, among which the 12q24 region showed the most significant evidence of linkage. GWLS studies identified Inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) gene in association with risk of KD (83).

Genome-Wide Association Studies (GWAS)

GWAS are based on the analysis of a genome-wide set of genetic variants typically SNP in affected individuals and healthy controls to see if a variant is repeatedly associated with a disease. GWAS studies on KD have identified several genes in association with the disease. The significant susceptibility genes identified include caspase-3 (CASP3) (69), calcium release-activated calcium modulator 1 (ORAI1) (59), ATP-binding cassette sub-family C member 4 gene(ABCC4) (84), Fc Fragment of IgG Receptor IIa (FCGR2A) (66), Transforming growth factor β pathway (TGFB2, TGFBR2, and SMAD3) (85), B lymphocyte kinase (BLK) (63), matrix metalloproteinase 11 (MMP-11) (74), and CD40 (86).

Genes Associated With B-Cell Signaling

CD40

CD40L–CD40 interaction is known in relation to triggering and progression of acute coronary syndromes (87). Higher CD40 ligand expression on CD4 T-cells of KD patients as compared to febrile controls has been reported by a previous study. This over-expression decreased after IVIg administration (88). SNPs around CD40 showed association with KD susceptibility in Japanese (rs4813003, located 4.9 Kb downstream) and Taiwanese (rs1569723, located 4.8 Kb upstream) population. GWAS from Taiwan and Japan have also shown the association of SNPs of *BLK* and *CD40* in the pathogenesis of KD (66).

B Lymphoid Tyrosine Kinase (BLK)

BLK has a role in signal transduction of B cells (64). GWAS from Taiwan and Japan have shown the association of SNPs of *BLK* and *CD40* in the pathogenesis of KD (68). Kawasaki Disease Genetics Consortium (2013) confirmed the association of BLK with KD susceptibility in Korean and European population (89).

Fc Fragment of IgG Receptor IIa (FCGR2A)

FCGR2A is present on Fc region of IgG and encodes for cell surface receptor protein on phagocytic cells. GWAS done in five independent centers have confirmed the association of *FCGR2A* with KD susceptibility (66). Associations of SNPs rs2736340, rs4813003, rs3818298, and rs1801274 with KD were reported in a Han Chinese population (60, 66).

T-Cell Activation Genes

Inositol 1,4,5-Trisphosphate 3-kinase (ITPK) Enzyme Gene

ITPKC is involved in Ca^{2+}/NFAT pathway and acts as a potential candidate for immune activation and T-cell receptor signaling (68, 83). Association of functional SNP (rs28493229) in ITPKC with development of KD and CAA risk was first reported in Japanese and American children (83). However, no significant association was found between rs28493229 and KD risk in Taiwanese patients (90). Significant associations of C-allele of rs28493229 with KD and BCG scar reactivation in the acute stage were reported by Lin et al. (56).

Calcium Release-Activated Calcium Channel Protein 1 (ORAI1)

ORAI1 is a Ca^{2+} channel protein involved in T cell regulation and inflammatory responses. Onouchi et al. (59) showed a significant non-synonymous association of SNP (rs3741596) in exon 2 of the ORAI1 gene with a high risk of KD in Japan. Another study reported a rare variant (rs141919534) in association with KD (91).

Genes Associated With the Apoptotic Pathway

Caspase 3 (CASP3)

CASP3 gene is an execution-phase caspase involved in apoptosis of immune cells. Various SNPs including rs2720378, rs72689236, and rs113420705 have been reported in association with KD (69). Kuo et al. found that SNP rs28493229 (*ITPKC*) and rs113420705 (*CASP3*) showed an increased risk of IVIG resistance and development of CAAs in Japanese but not in Taiwanese population (92).

TGF-β Signaling Pathway

The TGF-β pathway is an essential component of inflammation, T-cell activation and tissue remodeling. Genetic variations in *TGF-β2, TGF-βR2,* and *SMAD3* are associated with CAA formation and was confirmed in replication studies (85). SNPrs6550004 in TGF-βR2 and rs1495592 in SMAD5 showed significant associations with KD in the Korean population (93).

Gene Expression Studies

MicroRNAs (miRNAs) are 20–26 nucleotides long non-coding single-stranded RNA segments that modify post-transcriptional mRNA gene expression. Shimizu et al. (94) showed 6 miRNAs (miR-143, miR-199b-5p, miR-618, miR-223, miR-145, and miR-145) that were differentially expressed in acute KD. In another study, miRNA-200c and miRNA-371-5p have also been studied as diagnostic biomarkers of KD (54). miR-27b suppresses endothelial cell proliferation and has been studied as a therapeutic target in patients of acute KD (95).

- In a recent multicenter study by Wright et al. (96), the use of whole-blood gene expression patterns was explored and a 13-transcript blood gene expression signature has been developed to distinguish KD from other infectious and inflammatory febrile illnesses in the first week of illness.

- Whole genome sequencing in a family with KD has also shown genetic variations in the toll-like receptor-6 (TLR6) gene in their two affected children (97).

Summary: Most of the genetic studies have been carried out in small cohorts of patients with KD and the results are not reproducible across different populations and ethnicities. These studies require validations in larger and multinational cohorts with additional case-control sets for a better understanding of the genetic profiles.

CONCLUSIONS

The need for a robust set of biochemical biomarkers to validate the diagnosis of KD in the clinical setting has become the need of the hour. Clinical application of these biomarkers is limited. Inflammatory parameters can, at best, facilitate confirmation of a clinical diagnosis of KD but none of these is pathognomonic of KD. Further, these markers have very low specificity for the diagnosis of KD. The newer proteomic studies have identified some biomarkers in association with KD but these also need validation across different populations before these can be used for confirming a diagnosis of KD. Genome-wide studies, linkage studies and miRNA-based biomarkers are still in their early stage of development and fall short of being a diagnostic test for this enigmatic condition. These genetic markers are pointers toward a diagnostic panel for KD but clearly, these are early days and much more work needs to be done before a robust laboratory diagnostic test can be established.

AUTHOR CONTRIBUTIONS

HC, JN, RK: Literature review, interpretation of data and draft of the manuscript. VP: critical review and editing of the manuscript. DS: critical review of studies cited in the manuscript, editing of manuscript. AR: concept and design of the manuscript, critical review of studies cited in the manuscript, editing of manuscript. SS: concept and design of the manuscript, critical review, and editing of the manuscript.

REFERENCES

1. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics.* (1974) 54:271–6.
2. Lin M-T, Wu M-H. The global epidemiology of Kawasaki disease: review and future perspectives. *Glob Cardiol Sci Pract.* (2017) 2017:e201720. doi: 10.21542/gcsp.2017.20
3. Singh S, Bansal A, Gupta A, Kumar RM, Mittal BR. Kawasaki disease: a decade of experience from North India. *Int Heart J.* (2005) 46:679–89. doi: 10.1536/ihj.46.679
4. Khubchandani RP, Khemani C. Kawasaki disease registries reap results experience in Mumbai. *Indian J Pediatr.* (2006) 73:545–6. doi: 10.1007/BF02759910
5. Paul DK, Gupta A, Lahiri M. Kawasaki disease in Calcutta. *Indian Pediatr.* (2000) 37:1264–5.
6. Narayanan SN, Krishnaveni null, Sabarinathan K. Kawasaki disease. *Indian Pediatr.* (1997) 34:139–43.
7. Suresh N, Varadarajan VV, Ranjith MS. Kawasaki disease in south India: a prospective, case-control study. *Ann Trop Paediatr.* (2007) 27:277–83. doi: 10.1179/146532807X245661
8. Singh S, Jindal AK, Pilania RK. Diagnosis of Kawasaki disease. *Int J Rheum Dis.* (2018) 21:36–44. doi: 10.1111/1756-185X.13224
9. Rahbari-Manesh AA, Salamati P, Ghaforian S, Zekavat M. Relationship between ESR, CRP, platelet count and coronary artery disease in Kawasaki disease. *Iran J Pediatr.* (2005) 15:139–44. Available online at: https://www.sid.ir/en/journal/ViewPaper.aspx?id=38243
10. Huang M-Y, Gupta-Malhotra M, Huang J-J, Syu F-K, Huang T-Y. Acute-phase reactants and a supplemental diagnostic aid for Kawasaki disease. *Pediatr Cardiol.* (2010) 31:1209–13. doi: 10.1007/s00246-010-9801-y
11. Tremoulet AH, Jain S, Chandrasekar D, Sun X, Sato Y, Burns JC. Evolution of laboratory values in patients with Kawasaki disease. *Pediatr Infect Dis J.* (2011) 30:1022–6. doi: 10.1097/INF.0b013e31822d4f56
12. Koyanagi H, Yanagawa H, Nakamura Y, Yashiro M. Leukocyte counts in patients with Kawasaki disease: from the results of nationwide surveys of Kawasaki disease in Japan. *Acta Paediatr.* (1997) 86:1328–32. doi: 10.1111/j.1651-2227.1997.tb14907.x
13. Dominguez SR, Martin B, Heizer H, Jone P-N, Tong S, Davidson J, et al. Procalcitonin (PCT) and Kawasaki disease: does PCT correlate with IVIG-resistant disease, admission to the intensive care unit, or development of coronary artery lesions? *J Pediatr Infect Dis Soc.* (2016) 5:297–302. doi: 10.1093/jpids/piv019
14. Kuo H-C, Liang C-D, Wang C-L, Yu H-R, Hwang K-P, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. *Acta Paediatr.* (2010) 99:1578–83. doi: 10.1111/j.1651-2227.2010.01875.x
15. Roy S, Majumdar SD, Chakrabartty S, Chakravarti S. Mean platelet volume as a marker of Kawasaki disease in children. *Indian J Child Health.* (2017) 318–21. Available online at: https://www.researchgate.net/profile/Soumya_Roy11/publication/318392865_Mean_platelet_volume_as_a_marker_of_Kawasaki_disease_in_children/links/5966fb9da6fdcc18ea60a96e/Mean-platelet-volume-as-a-marker-of-Kawasaki-disease-in-children.pdf
16. Lee NH, Choi HJ, Kim YH. Clinical usefulness of serum procalcitonin level in distinguishing between Kawasaki disease and other infections in febrile children. *Korean J Pediatr.* (2017) 60:112–7. doi: 10.3345/kjp.2017.60.4.112
17. Öner T, Yilmazer MM, Güven B, Devrim I, Cilengiroglu ÖV, Demirpençe S, et al. An observational study on peripheral blood eosinophilia in incomplete Kawasaki disease. *Anatol J Cardiol.* (2012) 12:160–4. doi: 10.5152/akd.2012.042
18. Xiu-Yu S, Jia-Yu H, Qiang H, Shu-Hui D. Platelet count and erythrocyte sedimentation rate are good predictors of Kawasaki disease: ROC analysis. *J Clin Lab Anal.* (2010) 24:385–8. doi: 10.1002/jcla.20414
19. Liu R, Gao F, Huo J, Yi Q. Study on the relationship between mean platelet volume and platelet distribution width with coronary artery lesion in children with Kawasaki disease. *Platelets.* (2012) 23:11–6. doi: 10.3109/09537104.2011.586073
20. Jin J, Wang J, Lu Y, Fan Z, Huang N, Ma L, et al. Platelet-derived microparticles: a new index of monitoring platelet activation and inflammation in Kawasaki disease. *Indian J Pediatr.* (2018) 86:250–55. doi: 10.1007/s12098-018-2765-2
21. Tomoyuki Takahashi SK. Circulating myeloid dendritic cells is decreased in the acute phase of kawasaki disease. *J Clin Exp Cardiol.* (2013) 4:272. doi: 10.4172/2155-9880.1000272
22. Furukawa S, Matsubara T, Yabuta K. Mononuclear cell subsets and coronary artery lesions in Kawasaki disease. *Arch Dis Child.* (1992) 67:706–8. doi: 10.1136/adc.67.6.706
23. Brogan PA, Shah V, Clarke LA, Dillon MJ, Klein N. T cell activation profiles in Kawasaki syndrome. *Clin Exp Immunol.* (2008) 151:267–74. doi: 10.1111/j.1365-2249.2007.03567.x
24. Rivas MN, Lee Y, Wakita D, Chiba N, Dagvadorj J, Shimada K, et al. CD8+ T cells contribute to the development of coronary arteritis in the *Lactobacillus casei* extract-induced murine model of Kawasaki disease. *Arthritis Rheumatol.* (2017) 69:410–21. doi: 10.1002/art.39939

25. Wang Y, Wang W, Gong F, Fu S, Zhang Q, Hu J, et al. Evaluation of intravenous immunoglobulin resistance and coronary artery lesions in relation to Th1/Th2 cytokine profiles in patients with Kawasaki disease. *Arthritis Rheum.* (2013) 65:805–14. doi: 10.1002/art.37815
26. Brown TJ, Crawford SE, Cornwall ML, Garcia F, Shulman ST, Rowley AH. CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *J Infect Dis.* (2001) 184:940–3. doi: 10.1086/323155
27. Ehara H, Kiyohara K, Izumisawa Y, Ito T. Early activation does not translate into effector differentiation of peripheral CD8T cells during the acute phase of Kawasaki disease. *Cell Immunol.* (2010) 265:57–64. doi: 10.1016/j.cellimm.2010.07.003
28. Zhou Y, Wang S, Zhao J, Fang P. Correlations of complication with coronary arterial lesion with VEGF, PLT, D-dimer and inflammatory factor in child patients with Kawasaki disease. *Eur Rev Med Pharmacol Sci.* (2018) 22:5121–6. doi: 10.26355/eurrev_201808_15706
29. Sohn MH, Noh SY, Chang W, Shin KM, Kim DS. Circulating interleukin 17 is increased in the acute stage of Kawasaki disease. *Scand J Rheumatol.* (2003) 32:364–6. doi: 10.1080/03009740410005034
30. Lin K-H, Chang S-S, Yu C-W, Lin S-C, Liu S-C, Chao H, et al. Usefulness of natriuretic peptide for the diagnosis of Kawasaki disease: a systematic review and meta-analysis. *BMJ Open.* (2015) 5:6703. doi: 10.1136/bmjopen-2014-006703
31. Dionne A, Dahdah N. A decade of NT-proBNP in acute Kawasaki disease, from physiological response to clinical relevance. *Children.* (2018) 5:141. doi: 10.3390/children5100141
32. Shiraishi M, Fuse S, Mori T, Doyama A, Honjyo S, Hoshino Y, et al. N-terminal pro-brain natriuretic Peptide as a useful diagnostic marker of acute Kawasaki disease in children. *Circ J.* (2013) 77:2097–101. doi: 10.1253/circj.CJ-12-1281
33. Jia S, Li C, Wang G, Yang J, Zu Y. The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol.* (2010) 162:131–7. doi: 10.1111/j.1365-2249.2010.04236.x
34. Kimura J, Takada H, Nomura A, Ohno T, Mizuno Y, Saito M, et al. Th1 and Th2 cytokine production is suppressed at the level of transcriptional regulation in Kawasaki disease. *Clin Exp Immunol.* (2004) 137:444–9. doi: 10.1111/j.1365-2249.2004.02506.x
35. Ko T-M, Kuo H-C, Chang J-S, Chen S-P, Liu Y-M, Chen H-W, et al. CXCL10/IP-10 is a biomarker and mediator for Kawasaki disease. *Circ Res.* (2015) 116:876–83. doi: 10.1161/CIRCRESAHA.116.305834
36. Shikishima Y, Saeki T, Matsuura N. Chemokines in Kawasaki disease: measurement of CCL2, CCL22 and CXCL10. *Asian Pac J Allergy Immunol.* (2003) 21:139–43.
37. Hui-Yuen JS, Duong TT, Yeung RSM. TNF-α is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease. *J Immunol.* (2006) 176:6294–301. doi: 10.4049/jimmunol.176.10.6294
38. Kaneko K, Yoshimura K, Tsuji S. Brain natriuretic peptide as a novel diagnostic biomarker in Kawasaki disease. *J Compr Pediatr.* (2014) 5:19505. doi: 10.17795/compreped-19505
39. Kim SY, Han MY, Cha S-H, Jeon YB. N-terminal pro-brain natriuretic peptide (NT proBNP) as a predictive indicator of initial intravenous immunoglobulin treatment failure in children with Kawasaki disease: a retrospective study. *Pediatr Cardiol.* (2013) 34:1837–43. doi: 10.1007/s00246-013-0724-2
40. Kim MK, Song MS, Kim GB. Factors predicting resistance to intravenous immunoglobulin treatment and coronary artery lesion in patients with Kawasaki disease: analysis of the Korean nationwide multicenter survey from 2012 to 2014. *Korean Circ J.* (2018) 48:71–9. doi: 10.4070/kcj.2017.0136
41. Reddy M, Singh S, Rawat A, Sharma A, Suri D, Rohit MK. Pro-brain natriuretic peptide (ProBNP) levels in North Indian children with Kawasaki disease. *Rheumatol Int.* (2016) 36:551–9. doi: 10.1007/s00296-016-3430-6
42. Sato YZ, Molkara DP, Daniels LB, Tremoulet AH, Shimizu C, Kanegaye JT, et al. Cardiovascular biomarkers in acute Kawasaki disease. *Int J Cardiol.* (2013) 164:58–63. doi: 10.1016/j.ijcard.2011.06.065
43. Kim M, Kim K. Elevation of cardiac troponin I in the acute stage of Kawasaki disease. *Pediatr Cardiol.* (1999) 20:184–8. doi: 10.1007/s002469900437
44. Reindel R, Kim K-YA, Baker SC, Shulman ST, Perlman EJ, Lingen MW, et al. Periostin is upregulated in coronary arteriopathy in Kawasaki disease and is a potential diagnostic biomarker. *Pediatr Infect Dis J.* (2014) 33:659–61. doi: 10.1097/INF.0000000000000233
45. Yang S, Song R, Li X, Zhang T, Fu J, Cui X. Thrombospondin-2 predicts response to treatment with intravenous immunoglobulin in children with Kawasaki disease. *BMJ Paediatr Open.* (2018) 2:e000190. doi: 10.1136/bmjpo-2017-000190
46. Yu H-R, Kuo H-C, Huang E-Y, Liang C-D, Hwang K-P, Lin I-C, et al. Plasma clusterin levels in predicting the occurrence of coronary artery lesions in patients with Kawasaki disease. *Pediatr Cardiol.* (2010) 31:1151–6. doi: 10.1007/s00246-010-9769-7
47. Yu X, Hirono K-I, Ichida F, Uese K-I, Rui C, Watanabe S, et al. Enhanced iNOS expression in leukocytes and circulating endothelial cells is associated with the progression of coronary artery lesions in acute Kawasaki disease. *Pediatr Res.* (2004) 55:688–94. doi: 10.1203/01.PDR.0000113464.93042.A4
48. Yokouchi Y, Oharaseki T, Enomoto Y, Sato W, Imanaka-Yoshida K, Takahashi K. Expression of tenascin C in cardiovascular lesions of Kawasaki disease. *Cardiovasc Pathol.* (2018) 38:25–30. doi: 10.1016/j.carpath.2018.10.005
49. Kentsis A, Shulman A, Ahmed S, Brennan E, Monuteaux MC, Lee Y-H, et al. Urine proteomics for discovery of improved diagnostic markers of Kawasaki disease. *EMBO Mol Med.* (2013) 5:210–20. doi: 10.1002/emmm.201201494
50. 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) 22:216–21. doi: 10.2188/jea.JE20110126
51. Park YW, Han JW, Hong YM, Ma JS, Cha SH, Kwon TC, et al. Epidemiological features of Kawasaki disease in Korea, 2006-2008. *Pediatr Int.* (2011) 53:36–9. doi: 10.1111/j.1442-200X.2010.03178.x
52. Holman RC, Belay ED, Christensen KY, Folkema AM, Steiner CA, Schonberger LB. Hospitalizations for Kawasaki syndrome among children in the United States, 1997–2007. *Pediatr Infect Dis J.* (2010) 29:483–8. doi: 10.1097/INF.0b013e3181cf8705
53. Harnden A, Mayon-White R, Perera R, Yeates D, Goldacre M, Burgner D. Kawasaki disease in England: ethnicity, deprivation, and respiratory pathogens. *Pediatr Infect Dis J.* (2009) 28:21–4. doi: 10.1097/INF.0b013e3181812ca4
54. Fildes N, Burns JC, Newburger JW, Klitz W, Begovich AB. The HLA class II region and susceptibility to Kawasaki disease. *Tissue Antigens.* (1992) 39:99–101. doi: 10.1111/j.1399-0039.1992.tb01915.x
55. Yu H-R, Kuo H-C, Sheen J-M, Wang L, Lin I-C, Wang C-L, et al. A unique plasma proteomic profiling with imbalanced fibrinogen cascade in patients with Kawasaki disease. *Pediatr Allergy Immunol.* (2009) 20:699–707. doi: 10.1111/j.1399-3038.2008.00844.x
56. Lin M-T, Wang J-K, Yeh J-I, Sun L-C, Chen P-L, Wu J-F, et al. Clinical implication of the C allele of the ITPKC gene SNP rs28493229 in Kawasaki disease: association with disease susceptibility and BCG scar reactivation. *Pediatr Infect Dis J.* (2011) 30:148–52. doi: 10.1097/INF.0b013e3181f43a4e
57. Peng Q, Chen C, Zhang Y, He H, Wu Q, Liao J, et al. Single-nucleotide polymorphism rs2290692 in the 3'UTR of ITPKC associated with susceptibility to Kawasaki disease in a Han Chinese population. *Pediatr Cardiol.* (2012) 33:1046–53. doi: 10.1007/s00246-012-0223-x
58. Kuo H-C, Yang KD, Juo S-HH, Liang C-D, Chen W-C, Wang Y-S, et al. ITPKC single nucleotide polymorphism associated with the Kawasaki disease in a Taiwanese population. *PLoS ONE.* (2011) 6:e17370. doi: 10.1371/journal.pone.0017370
59. Onouchi Y, Fukazawa R, Yamamura K, Suzuki H, Kakimoto N, Suenaga T, et al. Variations in ORAI1 gene associated with Kawasaki disease. *PLoS ONE.* (2016) 11:e0145486. doi: 10.1371/journal.pone.0145486
60. Lou J, Zhong R, Shen N, Lu X, Ke J, Duan J, et al. Systematic confirmation study of GWAS-identified genetic variants for Kawasaki disease in a Chinese population. *Sci Rep.* (2015) 5:8194. doi: 10.1038/srep08194
61. Cheng S-C, Cheng Y-Y, Wu J-L. [Association between gene polymorphism of CD40 gene and coronary artery lesion in Kawasaki disease]. *Chin J Contemp Pediatr.* (2014) 16:1025–8.
62. 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) 44:517–21. doi: 10.1038/ng.2220
63. Chang C-J, Kuo H-C, Chang J-S, Lee J-K, Tsai F-J, Khor CC, et al. Replication and meta-analysis of GWAS identified susceptibility loci in Kawasaki disease confirm the importance of B lymphoid tyrosine kinase (BLK) in

disease susceptibility. *PLoS ONE.* (2013) 8:e72037. doi: 10.1371/journal.pone.0072037
64. Lee Y-C, Kuo H-C, Chang J-S, Chang L-Y, Huang L-M, Chen M-R, et al. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. *Nat Genet.* (2012) 44:522–5. doi: 10.1038/ng.2227
65. Duan J, Lou J, Zhang Q, Ke J, Qi Y, Shen N, et al. A genetic variant rs1801274 in FCGR2A as a potential risk marker for Kawasaki disease: a case-control study and meta-analysis. *PLoS ONE.* (2014) 9:e103329. doi: 10.1371/journal.pone.0103329
66. Khor CC, Davila S, Breunis WB, Lee Y-C, Shimizu C, Wright VJ, et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. *Nat Genet.* (2011) 43:1241–6. doi: 10.1038/ng.981
67. Taniuchi S, Masuda M, Teraguchi M, Ikemoto Y, Komiyama Y, Takahashi H, et al. Polymorphism of Fc gamma RIIa may affect the efficacy of gamma-globulin therapy in Kawasaki disease. *J Clin Immunol.* (2005) 25:309–13. doi: 10.1007/s10875-005-4697-7
68. Wang W, Lou J, Zhong R, Qi Y, Shen N, Lu X, et al. The roles of Ca2+/NFAT signaling genes in Kawasaki disease: single- and multiple-risk genetic variants. *Sci Rep.* (2014) 4:5208. doi: 10.1038/srep05208
69. Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. *Hum Mol Genet.* (2010) 19:2898–906. doi: 10.1093/hmg/ddq176
70. Kuo H-C, Yu H-R, Juo S-HH, Yang KD, Wang Y-S, Liang C-D, et al. CASP3 gene single-nucleotide polymorphism (rs72689236) and Kawasaki disease in Taiwanese children. *J Hum Genet.* (2011) 56:161–5. doi: 10.1038/jhg.2010.154
71. Choi YM, Shim KS, Yoon KL, Han MY, Cha SH, Kim SK, et al. Transforming growth factor beta receptor II polymorphisms are associated with Kawasaki disease. *Korean J Pediatr.* (2012) 55:18–23. doi: 10.3345/kjp.2012.55.1.18
72. Kuo H-C, Onouchi Y, Hsu Y-W, Chen W-C, Huang J-D, Huang Y-H, et al. Polymorphisms of transforming growth factor-β signaling pathway and Kawasaki disease in the Taiwanese population. *J Hum Genet.* (2011) 56:840–5. doi: 10.1038/jhg.2011.113
73. Peng Q, Deng Y, Yang X, Leng X, Yang Y, Liu H. Genetic variants of ADAM17 are implicated in the pathological process of Kawasaki disease and secondary coronary artery lesions via the TGF-β/SMAD3 signaling pathway. *Eur J Pediatr.* (2016) 175:705–13. doi: 10.1007/s00431-016-2696-8
74. Ban JY, Kim SK, Kang SW, Yoon KL, Chung J-H. Association between polymorphisms of matrix metalloproteinase 11 (MMP-11) and Kawasaki disease in the Korean population. *Life Sci.* (2010) 86:756–9. doi: 10.1016/j.lfs.2010.03.012
75. Holman RC, Christensen KY, Belay ED, Steiner CA, Effler PV, Miyamura J, et al. Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawai'i. *Hawaii Med J.* (2010) 69:194–7.
76. Fujita Y, Nakamura Y, Sakata K, Hara N, Kobayashi M, Nagai M, et al. Kawasaki disease in families. *Pediatrics.* (1989) 84:666–9.
77. Kato S, Kimura M, Tsuji K, Kusakawa S, Asai T, Juji T, et al. HLA antigens in Kawasaki disease. *Pediatrics.* (1978) 61:252–5.
78. Burns JC, Shimizu C, Shike H, Newburger JW, Sundel RP, Baker AL, et al. Family-based association analysis implicates IL-4 in susceptibility to Kawasaki disease. *Genes Immun.* (2005) 6:438–44. doi: 10.1038/sj.gene.6364225
79. Maggioli E, Boiocchi C, Zorzetto M, Mannarino S, Bossi G, Cuccia M. HLA class III genes involvement in Kawasaki disease: a case–control study in Caucasian population. *Int J Immunogenet.* (2014) 41:44–53. doi: 10.1111/iji.12077
80. Ichida KHF. Utility of TNF-α as a biomarker and the possibility of anti-TNF-α therapy for Kawasaki disease. *Pediatr Ther.* (2014) 5:1–6. doi: 10.4172/2161-0665.1000257
81. Huang F-Y, Lee Y-J, Chen M-R, Hsu C-H, Lin S-P, Sung T-C. Polymorphism of transmembrane region of MICA gene and Kawasaki disease. *Exp Clin Immunogenet.* (2000) 17:130–7. doi: 10.1159/000019132
82. Onouchi Y, Tamari M, Takahashi A, Tsunoda T, Yashiro M, Nakamura Y, et al. A genomewide linkage analysis of Kawasaki disease: evidence for linkage to chromosome 12. *J Hum Genet.* (2007) 52:179–90. doi: 10.1007/s10038-006-0092-3
83. 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. doi: 10.1038/ng.2007.59
84. Che D, Pi L, Fang Z, Xu Y, Cai M, Fu L, et al. abcc4 variants modify susceptibility to Kawasaki Disease in a Southern Chinese population. *Dis Markers.* (2018) 2018:1–7. doi: 10.1155/2018/8638096
85. Shimizu C, Oharaseki T, Takahashi K, Kottek A, Franco A, Burns JC. The role of TGF-β and myofibroblasts in the arteritis of Kawasaki disease. *Hum Pathol.* (2013) 44:189–98. doi: 10.1016/j.humpath.2012.05.004
86. Kuo H-C, Chao M-C, Hsu Y-W, Lin Y-C, Huang Y-H, Yu H-R, et al. CD40 gene polymorphisms associated with susceptibility and coronary artery lesions of Kawasaki disease in the Taiwanese population. *Sci World J.* (2012) 2012:1–5. doi: 10.1100/2012/520865
87. Aukrust P, Müller F, Ueland T, Berget T, Aaser E, Brunsvig A, et al. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina. Possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation.* (1999) 100:614–20. doi: 10.1161/01.CIR.100.6.614
88. Wang C-L, Wu Y-T, Liu C-A, Lin M-W, Lee C-J, Huang L-T, et al. Expression of CD40 ligand on CD4+ T-cells and platelets correlated to the coronary artery lesion and disease progress in Kawasaki disease. *Pediatrics.* (2003) 111:E140-147. doi: 10.1542/peds.111.2.e140
89. Lee J-K, Hong YM, Jang GY, Yun SW, Yu JJ, Yoon KL, et al. Consortium-based genetic studies of Kawasaki disease in Korea: Korean Kawasaki disease genetics consortium. *Korean Circ J.* (2015) 45:443. doi: 10.4070/kcj.2015.45.6.443
90. Chi H, Huang F-Y, Chen M-R, Chiu N-C, Lee H-C, Lin S-P, et al. ITPKC gene SNP rs28493229 and Kawasaki disease in Taiwanese children. *Hum Mol Genet.* (2010) 19:1147–51. doi: 10.1093/hmg/ddp586
91. Kuo HC, Lin YJ, Juo SHH, Hsu YW, Chen WC, Yang KD, et al. Lack of association between ORAI1/CRACM1 Gene Polymorphisms and Kawasaki disease in the Taiwanese children. *J Clin Immunol.* (2011) 31:650–5. doi: 10.1007/s10875-011-9524-8
92. Zhang W. Gene-Gene Associations with the susceptibility of Kawasaki disease and coronary artery lesions. *PLoS ONE.* (2015) 10:e0143056. doi: 10.1371/journal.pone.0143056
93. Li Z, Han D, Jiang J, Chen J, Tian L, Yang Z. Association of PECAM-1 gene polymorphisms with Kawasaki disease in Chinese children. *Dis Markers.* (2017) 2017:1–6. doi: 10.1155/2017/2960502
94. Yun KW, Lee JY, Yun SW, Lim IS, Choi ES. Elevated serum level of microRNA (miRNA)-200c and miRNA-371-5p in children with Kawasaki disease. *Pediatr Cardiol.* (2014) 35:745–52. doi: 10.1007/s00246-013-0846-6
95. Rong X, Ge D, Shen D, Chen X, Wang X, Zhang L, et al. miR-27b suppresses endothelial cell proliferation and migration by targeting Smad7 in Kawasaki disease. *Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol.* (2018) 48:1804–14. doi: 10.1159/000492354
96. Wright VJ, Herberg JA, Kaforou M, Shimizu C, Eleftherohorinou H, Shailes H, et al. Diagnosis of Kawasaki disease using a minimal whole-blood gene expression signature. *JAMA Pediatr.* (2018) 172:e182293–e182293. doi: 10.1001/jamapediatrics.2018.2293
97. Kim J, Shimizu C, Kingsmore SF, Veeraraghavan N, Levy E, Ribeiro dos Santos AM, et al. Whole genome sequencing of an African American family highlights toll like receptor 6 variants in Kawasaki disease susceptibility. *PLoS ONE.* (2017) 12:e0170977. doi: 10.1371/journal.pone.0170977

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

frontiers in Pediatrics

MINI REVIEW
published: 28 June 2019
doi: 10.3389/fped.2019.00244

Aetiological Significance of Infectious Stimuli in Kawasaki Disease

Akihiro Nakamura[1]*, *Kazuyuki Ikeda*[2] and *Kenji Hamaoka*[3,4]

[1] Central Research Laboratory, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan, [2] Department of Pediatrics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan, [3] Pediatric Cardiology and Kawasaki Disease Center, Uji-Tokushukai Medical Center, Kyoto, Japan, [4] Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Kawasaki disease (KD) is a pediatric vasculitis syndrome that is often involves coronary artery lesions (e. g., coronary artery aneurysms). Although its causal factors and entire pathogenesis remain elusive, the available evidence indicates that the pathogenesis of KD is closely associated with dysregulation of immune responses to various viruses or microbes. In this short review, we address several essential aspects of the etiology of KD with respect to the immune response to infectious stimuli: 1) the role of viral infections, 2) the role of bacterial infections and the superantigen hypothesis, 3) involvement of innate immune response including pathogens/microbe-associated molecular patterns and complement pathways, and 4) the influence of genetic background on the response to infectious stimuli. Based on the clinical and experimental evidence, we discuss the possibility that a wide range of microbes and viruses could cause KD through common and distinct immune processes.

Keywords: Kawasaki disease, vasculitis, animal models, infection, superantigens, pattern recognition receptors

OPEN ACCESS

Edited by:
Hiromichi Hamada,
Tokyo Women's Medical University Yachiyo Medical Center, Japan

Reviewed by:
Ying-Hsien Huang,
Kaohsiung Chang Gung Memorial Hospital, Taiwan
Hiroyuki Suzuki,
Wakayama Medical University, Japan

***Correspondence:**
Akihiro Nakamura
nakam993@koto.kpu-m.ac.jp

Specialty section:
This article was submitted to Pediatric Immunology, a section of the journal Frontiers in Pediatrics

Received: *31 December 2018*
Accepted: *29 May 2019*
Published: *28 June 2019*

Citation:
Nakamura A, Ikeda K and Hamaoka K (2019) Aetiological Significance of Infectious Stimuli in Kawasaki Disease. Front. Pediatr. 7:244. doi: 10.3389/fped.2019.00244

INTRODUCTION

Kawasaki disease (KD), named after its discoverer Dr. Tomisaku Kawasaki, is a pediatric vasculitis syndrome which is characterized by clinical manifestations including fever persisting for 5 days or more, swelling of the cervical lymph nodes, conjunctival infection, changes in oral mucosa and the tongue, skin rash, and redness of the palms and soles of the feet (1, 2). Although KD shows a systemic vascular inflammation, the coronary arteries are one of the worst affected sites. Without adequate treatment in the acute phase, approximately 30% of patients exhibit coronary artery lesions (CALs) including coronary arterial dilation, stenosis, and aneurysms (3).

Treatment of KD typically features intravenous immunoglobulin (IVIG) therapy (4). In fact, IVIG has markedly decreased the mortality rate in patients with acute KD. However, a persisting concern is that the disease may impair cardiovascular health in adults with a history of KD. Furthermore, approximately 20% of acute KD patients show a low response to IVIG (5). The therapeutic resistance also results in an increased risk of CALs and future cardiovascular events.

The aetiological mechanism of KD remains unclear and the causal factors are also unknown (6). Although there is no definitive evidence that KD is an infectious disease, recent studies support the view that a dysregulated immune response to a variety of infectious stimuli is likely to contribute to KD pathogenesis (6, 7). Based on these studies, this short review explores the possible relationship between KD and the immune response to various infectious agents (**Figure 1**).

Virus / Microbe	Possible effector molecule
Viruses	
Epstein-Barr virus [11,12]	dUTPase [15,123]
Unidentified RNA virus [25-27]	
Human corona virus HCoV-NL63 [121,122]	
Human immunodeficiency virus [124]	
Adenovirus [18,19]	
Human vocavirus (HBoV) [125]	
A variant of *Torque teno virus 7* [126]	
Human parvovirus B19 [127]	
Bacteria	
Staphyrococcus aureus [42,43]	TSST-1 [35]
Streptococcus pyrogenes [47]	SPEC [48]
Yersinia pseudotuberculosis [76]	YPMa [64]
Bacillus cereus [79]	
Mycoplasma pneumoniae [129]	
*Lactobacillus casei** [130]	β-glucan [128]
Mycobacterium ssp [67]	lipoarabinomannan [131]
Fungi	
Candida ssp [72]	
*Candida albicans** [81]	α-mannan [87,132]

FIGURE 1 | Possible causal microorganisms of Kawasaki disease. Most of the listed microorganisms were identified on the basis of PCR or serological examinations of clinical specimens. The asterisks indicate experimental evidence from animal models, not clinical specimens.

INVOLVEMENT OF VIRUSES IN KD PATHOGENESIS

The incidence of KD exhibits seasonality and outbreaks occurred in Japan in 1979, 1982, and 1986 (8, 9). This has led to the speculation that viral infection may underlie KD pathogenesis. Based on serological and polymerase chain reaction (PCR) based-analyses of clinical specimens, at least 14 species of the virus have been reported to be relevant to KD (10). We consider here three possible candidates: Epstein-Barr virus (EBV), human adenovirus, and a putative KD-associated RNA virus.

Epstein-Barr Virus

EBV is a type of human herpes virus. Kikuta et al. (11, 12) reported that the EBV DNA sequence was detected in 83% of KD patients and in 18% of control subjects. Chronic active EBV infection sometimes involves CALs, including coronary artery aneurysms (13, 14). Although the pathogenesis of EBV-infection-associated CALs is unclear, it has been demonstrated *in vitro* that deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase), an EBV-encoded protein, stimulates monocyte-derived macrophages through Toll-like receptor 2 (TLR2)-dependent signaling (15). This up-regulates the production of interleukin-6 (IL-6) (15), which activates endothelial cells (ECs) and platelets. Contrary evidence has also been presented on the involvement of EBV in KD (16, 17).

Adenovirus

It has been known that adenoviral infection exhibits seasonal pattern and some symptoms similar to that of KD (18, 19). However, semi-quantitative PCR-based investigations found no association between adenovirus and KD (20). Gene microarray of the blood samples also showed the distinct pattern between KD and adenovirus-infected patients (21). Further study is needed to clarify the involvement of adenoviral infection in KD (22).

Putative RNA-Associated Virus

Immunoglobulin A (IgA)-producing plasma cells are observed in the affected arterial tissue of KD patients (23, 24). Rowley et al. (25, 26) detected RNA virus-like inclusion bodies in the cytoplasm of bronchoepithelial cells of KD patients. It was detected using synthetic antibodies generated from the alpha and kappa chain-encoding DNA sequences, which were cloned from the affected arterial tissue of KD patients (27). However, the putative KD-associated RNA virus has not been identified yet.

Previous studies suggest that the involvement of viruses in KD is still very controversial. However, a recent transcriptomic study reported the significant up-regulation of a set of type I interferon (INF)-induced genes closely related to cellular antiviral processes in the coronary arteries of KD patients (28). Furthermore, the increased plasma level of C-X-C motif chemokine ligand 10 (CXCL10/IP-10), a representative INF-alpha2a/gamma-inducible protein was recently reported as a promising biomarker for the early acute phase of KD (29). These two studies raise the possibility that KD pathogenesis might be associated with a common immune response to viral infections.

BACTERIAL INFECTION AND SUPERANTIGENS HYPOTHESIS

Superantigens (SAgs) are a group of proteins, which can activate approximately 20% of the T cells in the peripheral blood. SAgs stimulate these cells by forming a bridge between the T-cell receptor and the major histocompatibility complex class II (MHC-II) of antigen presenting cells in the absence of any antigenic peptide presentation (30, 31). This results in the overproduction of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), by the activated T cells (32). Human MHC-class II and co-stimulators are also expressed in the endothelial cells (33). In fact, SAgs can directly injure these cells in conjunction with T cells (34, 35) *in vitro*.

SAgs are produced by a wide range of organisms, including bacteria, viruses, fungi, and plants (36–38). The pathological role of SAgs has been well-studied with respect to toxic shock syndrome and scarlet fever, which are caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, respectively (39, 40). KD displays some clinical similarities to these two diseases (41).

Staphylococcus aureus and *Streptococcus pyogenes*

Staphylococcus aureus produces a SAg designated TTS toxin-1 (TSST-1), which induces expansion of Vβ2 T cell receptor (TCR)-positive T cells. Some early studies reported the frequent detection of TSST-1-producing *S. aureus* or the specific antibody

to the SAg in KD-patients (42, 43). As ECs express MHC-II and co-stimulators, TSST-1 can activate human umbilical vein ECs *in vitro* in the presence of T cells (34). Considering that specific types of vessels (e.g., coronary arteries), are preferentially affected in KD., it might be worth investigating the pathophysiological significance of the role of ECs as a semi-professional antigen-presenting cells in KD (44, 45).

Streptococcal pyrogenic exotoxin C (SPEC) selectively activates Vβ2-bearing T-cell subsets and Vβ6.5-bearing ones (46). The number of Vβ2-/Vβ6.5-bearing T-cell subsets and anti-SPEC antibody levels are increased in the peripheral blood of acute KD patients (47). DNA fragments encoding these SAgs were also significantly more prevalent in the stool of KD patients than in that of non-KD febrile subjects (48), indicating that these bacteria-derived SAgs are involved in KD pathogenesis. *Streptococcus pyogenes* infection occasionally triggers autoimmune heart diseases, such as rheumatic fever. The serum level of IgM-type autoantibody to the cardiac myosin heavy chain, which is highly homologous to group A streptococcal M5 protein (49), was increased in KD patients (50). A variety of autoantibodies has also been detected in KD patients (51–53). Besides molecular mimicry, SAgs could be implicated in the activation of potentially autoreactive peripheral T and B cells (54).

Although the physiological role of circulating follicular T helper cells (cTfh) remains elusive, the cells could be stimulated by SAgs and/or other pathogen-derived molecules. Xu et al. (55) reported that IL-4 and the cTfh2 subpopulation of total cTfh cells significantly increased during the acute phase of KD. Increases in cTfh2 and IL-4 are observed in IgA-vasculitis and IgG4-related disease (IgG4-RD) (56–58). Although the general clinical features of IgG4-RD are apparently dissimilar to those of KD, IgG4-RD occasionally involves abnormalities of large- and intermediate-sized vessels, including coronary arteritis (59).

Despite the foregoing evidence, the involvement of SAgs in KD has not been definitively confirmed. Some studies have independently found no significant elevation of antibodies against *S. aureus* or *S. pyogenes*-derived SAgs in KD patients (60, 61). Although these bacterial-derived SAgs may provide a rationale to understand the pathogenesis of KD, its aetiological significance is still very controversial.

Yersinia pseudotuberculosis

Based on the similarity in clinical manifestations and the data from some serological studies, it has been argued that *Y. pseudotuberculosis* (YP) infections might be involved in the pathogenesis of KD (62). Some species of YP produce YP-derived mitogens (YPM), which are SAg-like virulence factors (63). Consistent with the increased prevalence of KD in Far East countries, YPM-positive pathogenic YP are also predominantly distributed in these countries and are less frequent in western countries (64). However, in a recent clinical study involving 108 Japanese KD patients, it was found that 90% of patients were negative for antibodies to YP and YPM (65). Vβ2-bearing T-cell subsets be preferentially activated in KD, whereas Vβ3, Vβ9, Vβ13.1, or Vβ13.2-bearing T-cell subsets are activated in YP infection (66).

Mycobacterium

Reactivation of *Bacillus Calmette-Guérin* (BCG) scar is a well-established clinical manifestation in acute KD, indicating that Mycobacteria or immunologically related pathogens might be involved in KD. Although Mycobacteria have not been isolated from KD patients as a causal pathogen, antibody and $CD4^+$/$CD8^+$ T-cell clones specific to Mycobacterium heat shock protein 65 have been detected in KD patients (67, 68).

Besides *M. tuberculosis, M. leprae,* and *M. lepromatosis*, non-tuberculous mycobacteria (NTM) can cause self-limited infections in humans. *M. avium complex* (MAC) is a representative NTM. The immune response to MAC infections involves peculiar macrophages, which are characterized by the co-expression of anti-inflammatory M2 macrophage markers (e.g., CD163, IL10) and markers of pro-inflammatory M1 macrophages (e.g., CCR7, IL1β) (69). These cells promote Th17 cell-differentiation (69). Intriguingly, the coronary arteries affected in acute KD also often feature marked infiltration of macrophages, which are negative for CD80 (an M1 marker), and positive for CD163 (an M2 marker) (70). Plasma level of Th17-related cytokine sets are also increased (71). Considering these recent findings and BCG scar reactivation in KD, re-visiting the possible implication of mycobacteria in KD could be warranted.

Fungi

Although there is no definitive clinical evidence suggesting that fungi are a causal factor for KD, it has been well-established that *Candida albicans* extracts develop KD-like experimental model of vasculitis in mice. Based on the meteorological and enviromentological study, Rodo et al. (72) recently reported that KD is associated with tropospheric winds containing Candida species as a predominant fungus. Similar investigation in Canada also suggested the implication of westerly wind-associated fungi in KD (73).

It might be possible to validate phlogogenic activity of the wind-blown microbes / molecules with established animal models for KD.

Possible Triggers and Diagnostic Criteria of Kawasaki Disease

Except *Y. pseudotuberculosis* infection (65, 74, 75), few cases satisfy the six diagnostic criteria for Kawasaki disease (KD) among the infectious diseases caused by the aforementioned agents. Considering that genetic background is closely associated with the susceptibility to KD (see section Influence of Genetic Background Affecting Response to Infectious Stimuli), polymorphisms in some specific genes of infected children might affect the clinical futures of the infectious diseases (76). Moreover, it is not exclusive that unidentified agents could be involved in the onset of KD. Caution should be exercised when considering the possible causal agents on the basis of the symptom similarity.

AETIOLOGICAL SIGNIFICANCE OF INNATE IMMUNE RESPONSE IN KD

While innate immunity is the first line of self-defense against infectious agents, it is also accompanied by inflammatory reaction (77). Thus, its inadequate regulation gives rise to inflammatory tissue damage. Accumulating evidence indicates that KD could be associated with dysregulated innate immune response.

Insight From Experimental Studies With Animal Model for KD

Apart from SAgs, a variety of microbes or virus-derived biomolecules (e.g., lipopolysaccharides, glucans, and nucleotides) can stimulate immune cells or other cell types, including ECs. These biomolecules are designated pathogen-associated molecular patterns (PAMPs) and microbe-associated molecular patterns (MAMPs). PAMPs and MAMPs are recognized by a type of cellular or soluble receptors, which are designated pattern recognition receptors (PRRs) (e.g., TLRs) to trigger innate immune responses, including the production of inflammatory cytokines through intracellular signaling pathways. A possible implication of these PAMPs or MAMPs in KD has been demonstrated in mouse models and more recently in clinical specimens from KD patients (78, 79).

KD-like vasculitis can be induced in rabbit, swine, and mouse models by various methods (80–83). The known genetic background and ease of genetic manipulation have made mouse models the preferred model to investigate molecular pathogenesis of KD and to discover its therapeutic targets (84–88).

In a murine model of vasculitis, *Candida albicans* water-soluble fraction (CAWS) is used as the inducer. Marked inflammatory change is observed in the aortic root, including the coronary arteries in CAWS-treated mice (89, 90). While CCR2 and GM-CSF play an indispensable role in its pathogenesis (89, 90), T and B cells are also involved in the vasculitis (89). Sensitivity and severity of CAWS-induced vasculitis apparently depend on mouse strain, suggesting that genetic background affects its pathogenesis (also see section Influence of Genetic Background Affecting Response to Infectious Stimuli).

Another KD-like murine coronary arteritis model involves induction by *Lactobacillus casei* cell wall extract (LCWE). TLR2-dependent signaling, ILβ-dependent signaling, and $CD8^+$ T cells (cytotoxic T cells) play a crucial role in its pathogenesis (91–93).

KD-like coronary arteritis can also be induced in mice by NOD1 ligand, FK565 (94, 95), a synthetic derivative of acylpeptide produced by *Streptomyces olivaceogriseus* (96). The experimental model of vasculitis reportedly underlies the interaction between the CCR2 expressed in monocytes and the CCL2 induced by the NOD1-dependent signaling in EC (78).

It is important to consider whether these models accurately represent the actual molecular mechanisms of KD (97). However, some evidences indicate that the experimental murine vasculitis is relevant to KD pathogenesis. The antibodies to β-glucan, another major CAWS component, was increased in KD patients (98). Regarding findings in CAWS-induced or FK565-induced mouse model, it was also reported that genetic polymorphisms in the CCR3-CCR2-CCR5 gene cluster are associated with KD susceptibility (99). Furthermore, it has been found that lipophilic substances almost identical to MAMPs derived from *Bacillus cereus, Bacillus subtilis, Y. pseudotuberculosis,* and *S. aureus*, have been detected in the serum of KD patients (79). This finding supports the possibility that KD underlies the molecular pathogenesis similar to the aforementioned mouse models. The possible involvement of *Y. pseudotuberculosis* and *S. aureus* in KD has been argued based on other clinical evidence (also see sections *Staphylococcus aureus* and *Streptococcus pyogenes* and *Yersinia pseudotuberculosis*). The collective findings indicate the possible relationship between PAMPs/MAMPs and the aetiological mechanism of KD vasculitis.

Other Insight to PAMPs/MAMPs Hypothesis in KD

Regarding the implication of PRRs, Huang et al. (100) reported that the CpG sites of TLR genes were reversibly hypomethylated in the peripheral whole blood cells of acute KD patients, resulting in upregulated expression of TLRs. This transient epigenetic change supposedly potentiates the TLR-dependent innate immune response. DNA demethylation in immune cells is also observed in patients infected with mycobacteria and certain viruses (101, 102), possibly implicating these intracellular-living pathogens in KD.

While killer immunoglobulin-like receptor (KIR) expressed in natural killer cells interacts with HLA-I to suppress activation of NK cells, KIR3DL1/2, a subtype of KIR, also recognizes and KIR3DL2 engulfs pathogen-derived CpG-oligodeoxynucleotide (CpG-ODN) to activate TLR9-dependent signaling (103). A recent case-control study of HLA-A and -B genotypes in Caucasian KD patients proposed the hypothesis that high abundance of HLA ligands for KIR3DL1/2 in KD patients interferes with KIR-dependent cellular CpG-ODN sensing to impair effective clearance of pathogens during infection (104). Consequently this impaired clearance might allow expansion of PAMPs/MAMPs. Although accumulating evidence suggests that PAMPs/MAMPs are involved in KD pathogenesis, their pathophysiological significance may be more complex than is currently appreciated.

Possible Implication of Complement Pathways

Three complement pathways (classical, lectin, and alternative pathway) are major components of the innate immune system against infectious agents. However, their excess activation causes inflammatory tissue injury. Indeed, accumulating evidence suggests that their dysregulated activation underlies the pathogenesis of vascular inflammation and aortic aneurysms (105, 106). Nevertheless, a limited number of studies had been undertaken regarding the involvement of complement pathways in KD (107, 108).

Recently Okuzaki et al. (109) found that ficolin-1, a circulating soluble PRR that is responsible for activating the lectin pathway,

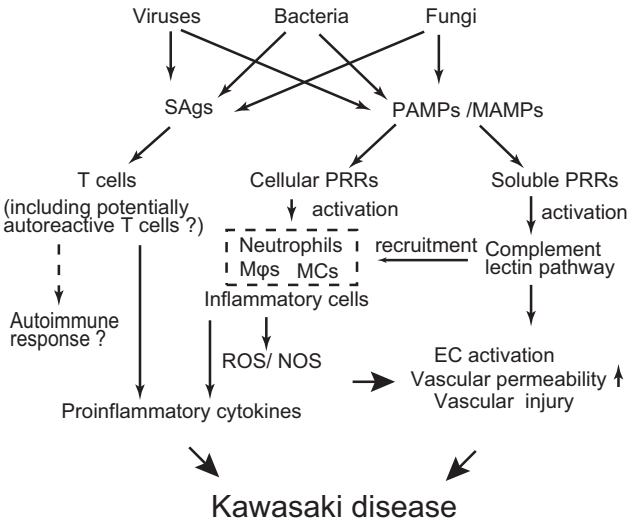

FIGURE 2 | Schematic depicting possible mechanisms of KD pathogenesis. Various infectious agents produce superantigens (SAgs) and pathogen/microbe-associated molecular patterns (PAMPs/MAMPs). SAgs non-specifically activate T cells, probably including potentially autoreactive T cells. PAMPs/MAMPs also stimulate immune cells [e.g., macrophages (Mϕ), dendritic cells (DCs), monocytes (MCs)] and endothelial cells (ECs) through cellular pattern recognition receptors (PRRs) (e.g., TLRs, NOD1, Dectin-1/-2). This stimulation up-regulates production of pro-inflammatory cytokines/chemokines and reactive oxygen/nitrogen species (ROS/NOS), leading to a systemic inflammatory reaction. On the other hand, PAMPs/MAMPs also activate the complement lectin pathway through soluble PRRs (e.g., ficolin-1, mannose binding lectin-2). Activated complement pathways can elicit inflammatory vascular damage through recruitment of innate inflammatory cells and direct injury to ECs. The extent of the inflammatory reaction is influenced by the genetic backgrounds of the individuals, resulting in a limited number of children developing KD in response to infectious stimuli.

was increased in acute KD. They also demonstrated that KD-like murine vasculitis is ameliorated by infusion of an inhibitory antibody to ficolin-1 (110), suggesting that the lectin pathway could participate in KD pathogenesis. The lectin pathway is triggered by activation of mannose-binding lectin-associated serine proteinases (MASPs). In addition to their role in the complement system, MASPs are involved in coagulation and EC activation (111), both of which are closely connected to KD.

Complement systems are rigorously regulated by more than ten protein species to prevent their undesirable excess activation. Genetic polymorphisms of these proteins might affect susceptibility of KD under infectious condition (106, 112).

INFLUENCE OF GENETIC BACKGROUND AFFECTING RESPONSE TO INFECTIOUS STIMULI

While a variety of microorganisms could be causative agents of KD, the prevalence of KD in children is potentially limited, suggesting that the genetic background of an individual likely affects the disease susceptibility. A series of clinical genetic investigations have revealed KD-related gene polymorphisms in more than 20 genes (113), although its aetiological significance is elucidated only in a limited number of these polymorphisms.

Statistical genetic studies identified an SNP associated with KD susceptibility and resistance to IVIG therapy in the gene encoding inositol-triphosphate 3-kinase C (ITPKC), which suppresses T-cell activation and regulates inflammasome activity in macrophages (114, 115). This SNP destabilizes ITPKC mRNA and reduces the cellular level of ITPKC protein (115). KD-associated gene polymorphisms have been discovered in the CASP3 gene, which might also influence the down-regulation of activated immune cells (116). In addition, Onouchi et al. (114) reported KD-associated polymorphisms in the gene encoding ORAI1, a calcium release-activated calcium channel protein 1. Notably, these proteins are also involved in the calcium-dependent nuclear factor-activated T cell (NFAT) pathway (117, 118).which regulates the function of T and B cells. EBV-encoded latent membrane protein 1 (LMP-1) up-regulates ORAI1 (119). Considering that EBV is a possible trigger for KD (see section Epstein-Barr Virus), it is intriguing whether the identified genetic polymorphisms could affect immune response to EBV infection.

The collective molecular genetic data indicate that most genes with KD-associated polymorphisms are responsible for the modulation of inflammatory responses, including T-cell activation, which is compatible with the postulated roles of SAg and PAMPs/MAMPs. This strengthens the possibility that the dysregulated immune response to infectious stimuli underlies the pathogenesis of KD.

Induced pluripotent stem cell technique has been successfully used to establish some human EC lines harboring genetic backgrounds of KD patients (120). Studies with these cells enable the verification of the aetiological significance of individual genetic backgrounds in KD *in vitro*.

CONCLUSION

Although the etiology of KD is far from being resolved, the evidence collected so far drives the following hypothesis (**Figure 2**): Diverse pathogens could be potential causative agents of KD. However, such different infectious stimuli converge on a similar/common immune process associated with the activation of T cells, innate immune cells, and ECs. The genetic background of infected children affects the magnitude of the immune responses to develop KD in a limited number of children.

AUTHOR CONTRIBUTIONS

The manuscript was written by AN, and it was edited by KI and KH.

REFERENCES

1. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi.* (1967) 16:178–222.
2. AHA Scientific Statement. Diagnostic guidelines for Kawasaki disease. *Circulation.* (2001) 103:335–6. doi: 10.1161/01.CIR.103.2.335
3. Kuo H. Preventing coronary artery lesions in Kawasaki disease. *Biomed J.* (2017) 40:141–6. doi: 10.1016/j.bj.2017.04.002
4. Lo MS, Newburger JW. Role of intravenous immunoglobulin in the treatment of Kawasaki disease. *Int J Rheum Dis.* (2018) 21:64–9. doi: 10.1111/1756-185X.13220
5. Uehara R, Belay ED, Maddox RA, Holman RC, Nakamura Y, Yashiro M, et al. Analysis of potential risk factors associated with nonresponse to initial intravenous immunoglobulin treatment among Kawasaki disease patients in Japan. *Pediatr Infect Dis J.* (2008) 27:155–60. doi: 10.1097/INF.0b013e31815922b5
6. Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. *Nat Rev Rheumatol.* (2015) 11:475–82. doi: 10.1038/nrrheum.2015.54
7. Chen KYH, Messina N, Germano S, Bonnici R, Freyne B, Cheung M, et al. Innate immune responses following Kawasaki disease and toxic shock syndrome. *PLoS ONE.* (2018) 13:e0191830. doi: 10.1371/journal.pone.0191830
8. Burns JC, Herzog L, Fabri O, Tremoulet AH, Rodó X, Uehara R, et al. Kawasaki disease global climate consortium. Seasonality of Kawasaki disease: a global perspective. *PLoS ONE.* (2013) 8:e74529. doi: 10.1371/journal.pone.0074529
9. 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) 22:216–21. doi: 10.2188/jea.JE20110126
10. Principi N, Rigante D, Esposito S. The role of infection in Kawasaki syndrome. *J Infect.* (2013) 67:1–10. doi: 10.1016/j.jinf.2013.04.004
11. Kikuta H, Taguchi Y, Tomizawa K, Kojima K, Kawamura N, Ishizaka A, et al. Epstein-Barr virus genome-positive T lymphocytes in a boy with chronic active EBV infection associated with Kawasaki-like disease. *Nature.* (1988) 333:455–7. doi: 10.1038/333455a0
12. Kikuta H, Matsumoto S, Yanase Y, Kawasaki T, Mizuno F, Osato T. Recurrence of Kawasaki disease and Epstein-Barr virus infection. *J Infect Dis.* (1990) 162:1215. doi: 10.1093/infdis/162.5.1215
13. Kikuta H, Sakiyama Y, Matsumoto S, Hamada I, Yazaki M, Iwaki T, et al. Detection of Epstein-Barr virus DNA in cardiac and aortic tissues from chronic, active Epstein-Barr virus infection associated with Kawasaki disease-like coronary artery aneurysms. *J Pediatr.* (1993) 123:90–2. doi: 10.1016/S0022-3476(05)81546-X
14. Muneuchi J, Ohga S, Ishimura M, Ikeda K, Yamaguchi K, Nomura A, et al. Cardiovascular complications associated with chronic active Epstein-Barr virus infection. *Pediatr Cardiol.* (2009) 30:274–81. doi: 10.1007/s00246-008-9343-8
15. Ariza ME, Glaser R, Kaumaya PT, Jones C, Williams MV. The EBV-encoded dUTPase activates NF-kappa B through the TLR2 and MyD88-dependent signaling pathway. *J Immunol.* (2009) 182:851–9. doi: 10.4049/jimmunol.182.2.851
16. Fuse S, Fujinaga E, Mori T, Hotsubo T, Kuroiwa Y, Morii M. Children with Kawasaki disease are not infected with Epstein-Barr virus. *Pediatr Infect Dis J.* (2010) 29:286–7. doi: 10.1097/INF.0b013e3181c3f111
17. Marchette NJ, Melish ME, Hicks R, Kihara S, Sam E, Ching D. Epstein-Barr virus and other herpesvirus infections in Kawasaki syndrome. *J Infect Dis.* (1990) 161:680–4. doi: 10.1093/infdis/161.4.680
18. Shike H, Shimizu C, Kanegaye JT, Foley JL, Schnurr DP, Wold LJ, et al. Adenovirus, adeno-associated virus and Kawasaki disease. *Pediatr Infect Dis J.* (2005) 24:1011–4. doi: 10.1097/01.inf.0000183769.31951.1e
19. Okano M, Thiele GM, Sakiyama Y, Matsumoto S, Purtilo DT. Adenovirus infection in patients with Kawasaki disease. *J Med Viol.* (1990) 32:53–7. doi: 10.1002/jmv.1890320109
20. Jaggi P, Kajon AE, Mejias A, Ramilo O, Leber A. Human adenovirus infection in Kawasaki disease: a confounding bystander? *Clin Infect Dis.* (2013) 56:58–64. doi: 10.1093/cid/cis807
21. Popper SJ, Watson VE, Shimizu C, Kanegaye JT, Burns JC, Relman DA. Gene transcript abundance profiles distinguish Kawasaki disease from adenovirus infection. *J Infect Dis.* (2009) 200:657–66. doi: 10.1086/603538
22. Song E, Kajon AE, Wang H, Salamon D, Texter K, Ramilo O, et al. Clinical and virologic characteristics may aid distinction of acute adenovirus disease from Kawasaki disease with incidental adenovirus detection. *J Pediatr.* (2016) 170:325–30. doi: 10.1016/j.jpeds.2015.11.021
23. Rowley AH, Shulman ST, Spike BT, Mask CA, Baker SC. Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. *J Immunol.* (2001) 166:1334–43. doi: 10.4049/jimmunol.166.2.1334
24. Rowley AH, Shulman ST, Mask CA, Finn LS, Terai M, Baker SC, et al. IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. *J Infect Dis.* (2000) 182:1183–91. doi: 10.1086/315832
25. Rowley AH, Baker SC, Orenstein JM, Shulman ST. Searching for the cause of Kawasaki disease–cytoplasmic inclusion bodies provide new insight. *Nat Rev Microbiol.* (2008) 6:394–401. doi: 10.1038/nrmicro1853
26. Rowley AH, Baker SC, Shulman ST, Garcia FL, Fox LM, Kos IM, et al. RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after acute Kawasaki disease. *PLoS ONE.* (2008) 3:e1582. doi: 10.1371/journal.pone.0001582
27. Rowley AH, Shulman ST, Garcia FL, Guzman-Cottrill JA, Miura M, Lee HL, et al. Cloning the arterial IgA antibody response during acute Kawasaki disease. *J Immunol.* (2005) 175:8386–91. doi: 10.4049/jimmunol.175.12.8386
28. Rowley AH, Wylie KM, Kim KY, Pink AJ, Yang A, Reindel R, et al. The transcriptional profile of coronary arteritis in Kawasaki disease. *BMC Genomics.* (2015) 16:1076. doi: 10.1186/s12864-015-2323-5
29. Ko TM, Kuo HC, Chang JS, Chen SP, Liu YM, Chen HW, et al. CXCL10/IP-10 is a biomarker and mediator for Kawasaki disease. *Circ Res.* (2015) 116:876–83. doi: 10.1161/CIRCRESAHA.116.305834
30. Sriskandan S, Faulkner L, Hopkins P. *Streptococcus pyogenes*: insight into the function of the *Streptococcal superantigens*. *Int J Biochem Cell Biol.* (2007) 39:12–9. doi: 10.1016/j.biocel.2006.08.009
31. Spaulding AR, Salgado-Pabón W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and *Streptococcal superantigen* exotoxins. *Clin Microbiol Rev.* (2013) 26:422–47. doi: 10.1128/CMR.00104-12
32. Faulkner L, Cooper A, Fantino C, Altmann DM, Sriskandan S. The mechanism of superantigen-mediated toxic shock: not a simple Th1 cytokine storm. *J Immunol.* (2005) 175:6870–7. doi: 10.4049/jimmunol.175.10.6870
33. Turesson C. Endothelial expression of MHC class II molecules in autoimmune disease. *Curr Pharm.* (2004) 10:129–43. doi: 10.2174/1381612043453414
34. Brogan PA, Shah V, Klein N, Dillon MJ. Vbeta-restricted T cell adherence to endothelial cells: a mechanism for superantigen-dependent vascular injury. *Arthritis Rheum.* (2004) 50:589–97. doi: 10.1002/art.20021
35. Kulhankova K, Kinney KJ, Stach JM, Gourronc FA, Grumbach IM, Klingelhutz AJ, et al. The superantigen toxic shock syndrome toxin-1 alters human aortic endothelial cell function. *Infect Immun.* (2017) 11:IAI.00848–17. doi: 10.1128/IAI.00848-17
36. Huber BT, Hsu PN, Sutkowski N. Virus-encoded superantigens. *Microbiol Rev.* (1996) 60:473–82.
37. Proft T, Fraser JD. Bacterial superantigens. *Clin Exp Immunol.* (2003) 133:299–306. doi: 10.1046/j.1365-2249.2003.02203.x
38. Devore-Carter D, Kar S, Vellucci V, Bhattacherjee V, Domanski P, Hostetter MK. Superantigen-like effects of a *Candida albicans* polypeptide. *J Infect Dis.* (2008) 197:981–9. doi: 10.1086/529203
39. Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol.* (1990) 17:251–72. doi: 10.3109/10408419009105728
40. O'Connell J, Sloand E. Kawasaki disease and *Streptococcal scarlet* fever. *J Nurse Pract.* (2013) 9:259–64. doi: 10.1016/j.nurpra.2012.12.013
41. Hall M, Hoyt L, Ferrieri P, Schlievert PM, Jenson HB. Kawasaki syndrome-like illness associated with infection caused by enterotoxin B-secreting *Staphylococcus aureus*. *Clin Infect Dis.* (1999) 29:586–9. doi: 10.1086/598638

42. Leung DY, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet.* (1993) 342:1385–8. doi: 10.1016/0140-6736(93)92752-F
43. Matsubara K, Fukaya T, Miwa K, Shibayama N, Nigami H, Harigaya H, et al. Development of serum IgM antibodies against superantigens of *Staphylococcus aureus* and *Streptococcus pyogenes* in Kawasaki disease. *Clin Exp Immunol.* (2006) 143:427–34. doi: 10.1111/j.1365-2249.2006.03015.x
44. Waisman A, Johann L. Antigen-presenting cell diversity for T cell reactivation in central nervous system autoimmunity. *J Mol Med.* (2018) 96:1279–92. doi: 10.1007/s00109-018-1709-7
45. Razakandrainibe R, Pelleau S, Grau GE, Jambou R. Antigen presentation by endothelial cells: what role in the pathophysiology of malaria? *Trends Parasitol.* (2012) 28:151–60. doi: 10.1016/j.pt.2012.01.004
46. Toyosaki T, Yoshioka T, Tsuruta Y, Yutsudo T, Iwasaki M, Suzuki R. Definition of the mitogenic factor (MF) as a novel streptococcal superantigen that is different from streptococcal pyrogenic exotoxins A, B, and C. *Eur J Immunol.* (1996) 26:2693–701. doi: 10.1002/eji.1830261122
47. Yoshioka T, Matsutani T, Iwagami S, Toyosaki-Maeda T, Yutsudo T, Tsuruta Y, Suzuki H, et al. Polyclonal expansion of TCRBV2- and TCRBV6-bearing T-cells in patients with Kawasaki disease. *Immunology.* (1999) 96:465–72. doi: 10.1046/j.1365-2567.1999.00695.x
48. Suenaga T, Suzuki H, Shibuta S, Takeuchi T, Yoshikawa N. Detection of multiple superantigen genes in stools of patients with Kawasaki disease. *J Pediatr.* (2009) 155:266–70. doi: 10.1016/j.jpeds.2009.03.013
49. Cunningham MW, Antone SM, Smart M, Liu R, Kosanke S. Molecular analysis of human cardiac myosin-cross-reactive B- and T-cell epitopes of the group A streptococcal M5 protein. *Infect Immun.* (1997) 65:3913–23.
50. Cunningham MW, Meissner HC, Heuser JS, Pietra BA, Kurahara DK, Leung DY. Anti-human cardiac myosin autoantibodies in Kawasaki syndrome. *J Immunol.* (1999) 163:1060–5.
51. Nussinovitch U, Shoenfeld Y. The clinical and diagnostic significance of anti-myosin autoantibodies in cardiac disease. *Clin Rev Allergy Immunol.* (2013) 44:98–108. doi: 10.1007/s12016-010-8229-8
52. Matsunaga A, Harita Y, Shibagaki Y, Shimizu N, Shibuya K, Ono H, et al. Identification of 4-trimethylaminobutyraldehyde dehydrogenase (TMABA-DH) as a candidate serum autoantibody target for Kawasaki disease. *PLoS ONE.* (2015) 10:e0128189. doi: 10.1371/journal.pone.0128189
53. Fujieda M, Karasawa R, Takasugi H, Yamamoto M, Kataoka K, Yudoh K, et al. A novel anti-peroxiredoxin autoantibody in patients with Kawasaki disease. *Microbiol Immunol.* (2012) 56:56–61. doi: 10.1111/j.1348-0421.2011.00393.x
54. Hurst JR, Kasper KJ, Sule AN, McCormick JK. *Streptococcal pharyngitis* and rheumatic heart disease: the superantigen hypothesis revisited. *Infect Genet Evol.* (2018) 61:160–75. doi: 10.1016/j.meegid.2018.03.006
55. Xu M, Jiang Y, Wang J, Liu D, Wang S, Yi H, et al. Distribution of distinct subsets of circulating T follicular helper cells in Kawasaki disease. *BMC Pediatr.* (2019) 19:43. doi: 10.1186/s12887-019-1412-z
56. Liu D, Liu J, Wang J, Guo L, Liu C, Jiang Y, et al. Distribution of circulating T follicular helper cell subsets is altered in immunoglobulin A vasculitis in children. *PLoS ONE.* (2017) 12:e0189133. doi: 10.1371/journal.pone.0189133
57. Rigante D, Castellazzi L, Bosco A, Esposito S. Is there a crossroad between infections, genetics and Henoch-Schönlein purpura? *Autoimmunity Rev.* (2013) 12:1016–21. doi: 10.1016/j.autrev.2013.04.003
58. Akiyama M, Yasuoka H, Yoshimoto K, Takeuchi T. Interleukin-4 contributes to the shift of balance of IgG subclasses toward IgG4 in IgG4-related disease. *Cytokine.* (2018) 110:416–9. doi: 10.1016/j.cyto.2018.05.009
59. Ishizaka N. IgG4-related disease underlying the pathogenesis of coronary artery disease. *Clin Chim Acta.* (2013) 415:20–5. doi: 10.1016/j.cca.2012.11.003
60. Morita A, Imada Y, Igarashi H, Yutsudo T. Serologic evidence that streptococcal superantigens are not involved in the pathogenesis of Kawasaki disease. *Microbiol Immunol.* (1997) 41:895–900. doi: 10.1111/j.1348-0421.1997.tb01947.x
61. Gupta-Malhotra M, Viteri-Jackson A, Thomas W, Zabriskie JB. Antibodies to highly conserved peptide sequence of staphylococcal and streptococcal superantigens in Kawasaki disease. *Exp Mol Pathol.* (2004) 76:117–21. doi: 10.1016/j.yexmp.2003.12.003
62. Vincent P, Salo E, Skurnik M, Fukushima H, Simonet M. Similarities of Kawasaki disease and Yersinia pseudotuberculosis infection epidemiology. *Pediatric Infect Dis J.* (2007) 26:629–31. doi: 10.1097/INF.0b013e3180616d3c
63. Donadini R, Fields BA. Yersinia pseudotuberculosis superantigens. *Chem Immunol Allergy.* (2007) 93:77–91. doi: 10.1159/000100859
64. Fukushima H, Matsuda Y, Seki R, Tsubokura M, Takeda N, Shubin FN, et al. Geographical heterogeneity between Far Eastern and Western countries in prevalence of the virulence plasmid, the superantigen Yersinia pseudotuberculosis-derived mitogen, and the high-pathogenicity island among Yersinia pseudotuberculosis strains. *J Clin Microbiol.* (2001) 39:3541–7. doi: 10.1128/JCM.39.10.3541-3547.2001
65. Horinouchi T, Nozu K, Hamahira K, Inaguma Y, Abe J, Nakajima H, et al. Yersinia pseudotuberculosis infection in Kawasaki disease and its clinical characteristics. *BMC Pediatr.* (2015) 15:177. doi: 10.1186/s12887-015-0497-2
66. Goubard A, Loïez C, Abe J, Fichel C, Herwegh S, Faveeuw C, et al. Superantigenic Yersinia pseudotuberculosis induces the expression of granzymes and perforin by CD4+ T cells. *Infect Immun.* (2015) 83:2053–64. doi: 10.1128/IAI.02339-14
67. Yokota S, Tsubaki K, Kuriyama T, Shimizu H, Ibe M, Mitsuda T, et al. Presence in Kawasaki disease of antibodies to mycobacterial heat-shock protein HSP65 and autoantibodies to epitopes of human HSP65 cognate antigen. *Clin Immunol Immunopathol.* (1993) 67:163–70. doi: 10.1006/clin.1993.1060
68. Sireci G, Dieli F, Salerno A. T cells recognize an immunodominant epitope of heat shock protein 65 in Kawasaki disease. *Mol Med.* (2000) 6:581–90. doi: 10.1007/BF03401796
69. Tatano Y, Shimizu T, Tomioka H. Unique macrophages different from M1/M2 macrophages inhibit T cell mitogenesis while upregulating Th17 polarization. *Sci Rep.* (2014) 4:4146. doi: 10.1038/srep04146
70. Takahashi K, Oharaseki T, Yokouchi Y. Histopathological aspects of cardiovascular lesions in Kawasaki disease. *Int J Rheumatic Dis.* (2018) 21:31–5. doi: 10.1111/1756-185X.13207
71. Guo MM, Tseng WN, Ko CH, Pan HM, Hsieh KS, Kuo HC. Th17- and Treg-related cytokine and mRNA expression are associated with acute and resolving Kawasaki disease. *Allergy.* (2015) 70:310–8. doi: 10.1111/all.12558
72. Rodó X, Curcoll R, Robinson M, Ballester J, Burns JC, Cayan DR, et al. Tropospheric winds from northeastern China carry the etiologic agent of Kawasaki disease from its source to Japan. *Proc Natl Acad Sci USA.* (2014) 111:7952–7. doi: 10.1073/pnas.1400380111
73. Manlhiot C, Mueller B, O'Shea S, Majeed H, Bernknopf B, Labelle M, et al. Environmental epidemiology of Kawasaki disease: Linking disease etiology, pathogenesis and global distribution. *PLoS ONE.* (2018) 13:e0191087. doi: 10.1371/journal.pone.0191087
74. Baba K, Takeda N, Tanaka M. Cases of *Yersinia pseudotuberculosis* infection having diagnostic criteria of Kawasaki disease. *Contrib Microbiol Immunol.* (1991) 12:292–6.
75. Sato K. *Yersinia pseudotuberculosis* infection in children. *Contrib Microbiol Immunol.* (1987) 9:111–6.
76. Vollmer-Conna U, Piraino BF, Cameron B, Davenport T, Hickie I, Wakefield D, et al. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. *Clin Infect Dis.* (2008) 47:1418–25. doi: 10.1086/592967
77. Xiao TS. Innate immunity and inflammation. *Cell Mol Immunol.* (2017) 14:1–3. doi: 10.1038/cmi.2016.45
78. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. *Clin Exp Immunol.* (2016) 186:134–43. doi: 10.1111/cei.12832
79. Kusuda T, Nakashima Y, Murata K, Kanno S, Nishio H, Saito M, et al. Kawasaki disease-specific molecules in the sera are linked to microbe-associated molecular patterns in the biofilms. *PLoS ONE.* (2014) 9:e113054. doi: 10.1371/journal.pone.0113054
80. Hamaoka-Okamoto A, Suzuki C, Yahata T, Ikeda K, Nagi-Miura N, Ohno N, et al. The involvement of the vasa vasorum in the development of vasculitis in animal model of Kawasaki disease. *Pediatr Rheumatol Online J.* (2014) 12:12. doi: 10.1186/1546-0096-12-12
81. Ohno N. Chemistry and biology of angiitis inducer, *Candida albicans* water-soluble mannoprotein-beta-glucan complex (CAWS). *Microbiol Immunol.* (2003) 47:479–90. doi: 10.1111/j.1348-0421.2003.tb03409.x

82. Fujii M, Tanaka H, Nakamura A, Suzuki C, Harada Y, Takamatsu T, et al. Histopathological characteristics of post-inflamed coronary arteries in Kawasaki disease-like vasculitis of rabbits. *Acta Histochem Cytochem.* (2016) 49:29–36. doi: 10.1267/ahc.15028
83. Philip S, Lee WC, Wu MH, Mammen CK, Lue HC. Histopathological evaluation of horse serum-induced immune complex vasculitis in swine: implication to coronary artery lesions in Kawasaki disease. *Pediatr Neonatol.* (2014) 55:297–305. doi: 10.1016/j.pedneo.2013.10.012
84. Yoshikane Y, Koga M, Imanaka-Yoshida K, Cho T, Yamamoto Y, Yoshida T, et al. JNK is critical for the development of *Candida albicans*-induced vascular lesions in a mouse model of Kawasaki disease. *Cardiovasc Pathol.* (2015) 24:33–40. doi: 10.1016/j.carpath.2014.08.005
85. Miyabe C, Miyabe Y, Miura NN, Takahashi K, Terashima Y, Toda E, et al. Am80, a retinoic acid receptor agonist, ameliorates murine vasculitis through the suppression of neutrophil migration and activation. *Arthritis Rheum.* (2013) 65:503–12. doi: 10.1002/art.37784
86. Suzuki C, Nakamura A, Miura N, Fukai K, Ohno N, Yahata T, et al. Non-receptor type, proline-rich protein tyrosine kinase 2 (Pyk2) is a possible therapeutic target for Kawasaki disease. *Clin Immunol.* (2017) 179:17–24. doi: 10.1016/j.clim.2017.01.013
87. Hirata N, Ishibashi K, Sato W, Nagi-Miura N, Adachi Y, Ohta S, et al. β-mannosyl linkages inhibit CAWS arteritis by negatively regulating dectin-2-dependent signaling in spleen and dendritic cells. *Immunopharmacol Immunotoxicol.* (2013) 35:594–604. doi: 10.3109/08923973.2013.830124
88. Matundan HH, Sin J, Rivas MN, Fishbein MC, Lehman TJ, Chen S, et al. Myocardial fibrosis after adrenergic stimulation as a long-term sequela in a mouse model of Kawasaki disease vasculitis. *JCI Insight.* (2019) 4:126279. doi: 10.1172/jci.insight.126279
89. Martinez HG, Quinones MP, Jimenez F, Estrada C, Clark KM, Suzuki K, et al. (2012) Important role of CCR2 in a murine model of coronary vasculitis. *BMC Immunol.* 13:56. doi: 10.1186/1471-2172-13-56
90. Stock AT, Hansen JA, Sleeman MA, McKenzie BS, Wicks IP. GM-CSF primes cardiac inflammation in a mouse model of Kawasaki disease. *J Exp Med.* (2016) 213:1983–98. doi: 10.1084/jem.20151853
91. Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, et al. Interleukin-1β is crucial for the induction of coronary artery inflammation in a mouse model of Kawasaki disease. *Circulation.* (2012) 125:1542–50. doi: 10.1161/CIRCULATIONAHA.111.072769
92. Rosenkranz ME, Schulte DJ, Agle LM, Wong MH, Zhang W, Ivashkiv L, et al. TLR2 and MyD88 contribute to Lactobacillus casei extract-induced focal coronary arteritis in a mouse model of Kawasaki disease. *Circulation.* (2005) 112:2966–73. doi: 10.1161/CIRCULATIONAHA.105.537530
93. Noval Rivas M, Lee Y, Wakita D, Chiba N, Dagvadorj J, Shimada K, et al. CD8+ T cells contribute to the development of coronary arteritis in the *Lactobacillus casei* cell wall extract-induced murine model of Kawasaki disease. *Arthritis Rheumatol.* (2017) 69:410–21. doi: 10.1002/art.39939
94. Nishio H, Kanno S, Onoyama S, Ikeda K, Tanaka T, Kusuhara K, et al. Nod1 ligands induce site-specific vascular inflammation. *Arterioscler Thromb Vasc Biol.* (2011) 31:1093–9. doi: 10.1161/ATVBAHA.110.216325
95. Motomura Y, Kanno S, Asano K, Tanaka M, Hasegawa Y, Katagiri H, et al. Identification of pathogenic cardiac CD11c+ macrophages in Nod1-mediated acute coronary arteritis. *Arterioscler Thromb Vasc Biol.* (2015) 35:1423–33. doi: 10.1161/ATVBAHA.114.304846
96. Kitaura Y, Nakaguchi O, Takeno H, Okada S, Yonishi S, Hemmi K, et al. N2-(gamma-D-Glutamyl)-meso-2(L),2'(D)-diaminopimelic acid as the minimal prerequisite structure of FK-156: its acyl derivatives with potent immunostimulating activity. *J Med Chem.* (1982) 25:335–7. doi: 10.1021/jm00346a001
97. Osterburg AR, Hexley P, Supp DM, Robinson CT, Noel G, Ogle C, et al. Concerns over interspecies transcriptional comparisons in mice and humans after trauma. *Proc Natl Acad Sci USA.* (2013) 110:E3370. doi: 10.1073/pnas.1306033110
98. Ishibashi K, Fukazawa R, Miura NN, Adachi Y, Ogawa S, Ohno N. Diagnostic potential of antibody titres against *Candida* cell wall β-glucan in Kawasaki disease. *Clin Exp Immunol.* (2014) 177:161–7. doi: 10.1111/cei.12328
99. Breunis WB, Biezeveld MH, Geissler J, Kuipers IM, Lam J, Ottenkamp J, et al. Polymorphisms in chemokine receptor genes and susceptibility to Kawasaki disease. *Clin Exp Immunol.* (2007) 150:83–90. doi: 10.1111/j.1365-2249.2007.03457.x
100. Huang YH, Li SC, Huang LH, Chen PC, Lin YY, Lin CC, et al. Identifying genetic hypomethylation and upregulation of Toll-like receptors in Kawasaki disease. *Oncotarget.* (2017) 8:11249–58. doi: 10.18632/oncotarget.14497
101. Pacis A, Tailleux L, Morin AM, Lambourne J, MacIsaac JL, Yotova V, et al. Bacterial infection remodels the DNA methylation landscape of human dendritic cells. *Genome Res.* (2015) 25:1801–11. doi: 10.1101/gr.192005.115
102. Hernando H, Shannon-Lowe C, Islam AB, Al-Shahrour F, Rodríguez-Ubreva J, Rodríguez-Cortez VC, et al. The B cell transcription program mediates hypomethylation and overexpression of key genes in Epstein-Barr virus-associated proliferative conversion. *Genome Biol.* (2013) 14:R3. doi: 10.1186/gb-2013-14-1-r3
103. Sivori S, Falco M, Carlomagno S, Romeo E, Soldani C, Bensussan A, et al. A novel KIR-associated function: evidence that CpG DNA uptake and shuttling to early endosomes is mediated by KIR3DL2. *Blood.* (2010) 116:1637–47. doi: 10.1182/blood-2009-12-256586
104. Capittini C, Emmi G, Mannarino S, Bossi G, Dellepiane RM, Salice P, et al. An immune-molecular hypothesis supporting infectious aetiopathogenesis of Kawasaki disease in children. *Eur J Immunol.* (2018) 48:543–5. doi: 10.1002/eji.201747226
105. Chimenti MS, Ballanti E, Triggianese P, Perricone R. Vasculitides and the complement system. *Clin Rev Allergy Immunol.* (2015) 49:333–46. doi: 10.1007/s12016-014-8453-8
106. Zhou HF, Yan H, Bertram P, Hu Y, Springer LE, Thompson RW, et al. Fibrinogen-specific antibody induces abdominal aortic aneurysm in mice through complement lectin pathway activation. *Proc Natl Acad Sci USA.* (2013) 110:E4335–44. doi: 10.1073/pnas.1315512110
107. Kohsaka T, Abe J, Asahina T, Kobayashi N. Classical pathway complement activation in Kawasaki syndrome. *J Allergy Clin Immunol.* (1994) 93:520–5. doi: 10.1016/0091-6749(94)90362-X
108. Biezeveld MH, Geissler J, Weverling GJ, Kuipers IM, Lam J, Ottenkamp J, et al. Polymorphisms in the mannose-binding lectin gene as determinants of age-defined risk of coronary artery lesions in Kawasaki disease. *Arthritis Rheum.* (2006) 54:369–76. doi: 10.1002/art.21529
109. Okuzaki D, Ota K, Takatsuki SI, Akiyoshi Y, Naoi K, Yabuta N, et al. FCN1 (M-ficolin), which directly associates with immunoglobulin G, is a molecular target of intravenous immunoglobulin therapy for Kawasaki disease. *Sci Rep.* (2017) 7:11334. doi: 10.1038/s41598-017-11108-0
110. Katayama M, Ota K, Nagi-Miura N, Ohno N, Yabuta N, Nojima H, et al. Ficolin-1 is a promising therapeutic target for autoimmune diseases. *Int Immunol.* (2019) 6:23–32. doi: 10.1093/intimm/dxy056
111. Dobó J, Pál G, Cervenak L, Gál P. The emerging roles of mannose-binding lectin-associated serine proteases (MASPs) in the lectin pathway of complement and beyond. *Immunol Rev.* (2016) 274:98–111. doi: 10.1111/imr.12460
112. Sato S, Kawashima H, Kashiwagi Y, Fujioka T, Takekuma K, Hoshika A. Association of mannose-binding lectin gene polymorphisms with Kawasaki disease in the Japanese. Association of mannose-binding lectin gene polymorphisms with Kawasaki disease in the Japanese. *Int J Rheumatol.* (2009) 12:307–10. doi: 10.1111/j.1756-185X.2009.01428.x
113. Onouchi Y. Molecular genetics of Kawasaki disease. *Pediatr Res.* (2009) 65 (5 Pt 2):46R−54R. doi: 10.1203/PDR.0b013e31819dba60
114. 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. doi: 10.1038/ng.2007.59
115. Alphonse MP, Duong TT, Shumitzu C, Hoang TL, McCrindle BW, Franco A, et al. Inositol-triphosphate 3-kinase C mediates inflammasome activation and treatment response in Kawasaki disease. *J Immunol.* (2016) 197:3481–9. doi: 10.4049/jimmunol.1600388
116. Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. *Hum Mol Genet.* (2010) 19:2898–906. doi: 10.1093/hmg/ddq176
117. Onouchi Y, Fukazawa R, Yamamura K, Suzuki H, Kakimoto N, Suenaga T, et al. Variations in ORAI1 gene associated with Kawasaki disease. *PLoS ONE.* (2016) 11:e0145486. doi: 10.1371/journal.pone.0145486

118. Srikanth S, Gwack Y. Orai1-NFAT signalling pathway triggered by T cell receptor stimulation. *Mol Cells.* (2013) 35:182–94. doi: 10.1007/s10059-013-0073-2
119. Dellis O, Arbabian A, Papp B, Rowe M, Joab I, Chomienne C. Epstein-Barr virus latent membrane protein 1 increases calcium influx through store-operated channels in B lymphoid cells. *J Biol Chem.* (2011) 286:18583–92. doi: 10.1074/jbc.M111.222257
120. Ikeda K, Mizoro Y, Ameku T, Nomiya Y, Mae SI, Matsui S, et al. Transcriptional analysis of intravenous immunoglobulin resistance in Kawasaki disease using an induced pluripotent stem cell disease model. *Circ J.* (2016) 81:110–8. doi: 10.1253/circj.CJ-16-0541
121. Dominguez SR, Anderson MS, Glodé MP, Robinson CC, Holmes KV. Blinded case-control study of the relationship between human coronavirus NL63 and Kawasaki syndrome. *J Infect Dis.* (2006) 194:1697–701. doi: 10.1086/509509
122. Chang LY, Chiang BL, Kao CL, Wu MH, Chen PJ, Berkhout B, et al. Kawasaki Disease Research Group. Lack of association between infection with a novel human coronavirus (HCoV), HCoV-NH, and Kawasaki disease in Taiwan. *J Infect Dis.* (2006) 193:283–6. doi: 10.1086/498875
123. Williams MV, Cox B, Ariza ME. Herpesviruses dUTPases: a new family of pathogen-associated molecular pattern (PAMP) proteins with implications for human disease. *Pathogens.* (2016) 6:E2. doi: 10.3390/pathogens6010002
124. Johnson RM, Bergmann KR, Manaloor JJ, Yu X, Slaven JE, Kharbanda AB. Pediatric Kawasaki disease and adult human immunodeficiency virus kawasaki-like syndrome are likely the same malady. *Open Forum Infect Dis.* (2016) 3:ofw160. doi: 10.1093/ofid/ofw160
125. Bajolle F, Meritet JF, Rozenberg F, Chalumeau M, Bonnet D, Gendrel D, et al. Markers of a recent bocavirus infection in children with Kawasaki disease: "a year prospective study". *Pathol Biol.* (2014) 62:356–8. doi: 10.1016/j.patbio.2014.06.002
126. Thissen JB, Isshiki M, Jaing C, Nagao Y, Lebron Aldea D, Allen JE, et al. A novel variant of torque teno virus 7 identified in patients with Kawasaki disease. *PLoS ONE.* (2018) 13:e0209683. doi: 10.1371/journal.pone.0209683
127. Nigro G, Zerbini M, Krzysztofiak A, Gentilomi G, Porcaro MA, Mango T, et al. Active or recent parvovirus B19 infection in children with Kawasaki disease. *Lancet.* (1994) 343:1260–1. doi: 10.1016/S0140-6736(94)92154-7
128. Lin IC, Suen JL, Huang SK, Huang SC, Huang HC, Kuo HC, et al. Dectin-1/Syk signaling is involved in *Lactobacillus casei* cell wall extract-induced mouse model of Kawasaki disease. *Immunobiology.* (2013) 218:201–12. doi: 10.1016/j.imbio.2012.04.004
129. Vitale EA, La Torre F, Calcagno G, Infricciori G, Fede C, Conti G, et al. Mycoplasma pneumoniae: a possible trigger of kawasaki disease or a mere coincidental association? Report of the first four Italian cases. *Minerva Pediatr.* (2010) 62:605–7.
130. Lehman TJ, Mahnovski V. Animal models of vasculitis. Lessons we can learn to improve our understanding of Kawasaki disease. *Rheum Dis Clin North Am.* (1998) 14:479–87.
131. Yonekawa A, Saijo S, Hoshino Y, Miyake Y, Ishikawa E, Suzukawa M, et al. Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. *Immunity.* (2014) 41:402–13. doi: 10.1016/j.immuni.2014.08.005
132. Oharaseki T, Yokouchi Y, Enomoto Y, Sato W, Ishibashi K, Miura N, et al. Recognition of alpha-mannan by dectin 2 is essential for onset of Kawasaki disease-like murine vasculitis induced by *Candida albicans* cell-wall polysaccharide. *Mod Rheumatol.* (2019) 29:1–8. doi: 10.1080/14397595.2019.1601852

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

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 09 August 2019
doi: 10.3389/fped.2019.00326

A Metagenomics Study on Hirschsprung's Disease Associated Enterocolitis: Biodiversity and Gut Microbial Homeostasis Depend on Resection Length and Patient's Clinical History

Alessio Pini Prato[1,2], Casey Bartow-McKenney[3], Kelly Hudspeth[4,5], Manuela Mosconi[2], Valentina Rossi[2], Stefano Avanzini[2], Maria G. Faticato[2,6], Isabella Ceccherini[7], Francesca Lantieri[8], Girolamo Mattioli[2,6], Denise Larson[9], William Pavan[9], Carlotta De Filippo[10], Monica Di Paola[11], Domenico Mavilio[4,5] and Duccio Cavalieri[11]**

[1] Division of Pediatric Surgery, AON SS Antonio e Biagio e Cesare Arrigo, Alessandria, Italy, [2] Division of Pediatric Surgery, Giannina Gaslini Institute, Genoa, Italy, [3] Department of Dermatology and Microbiology, University of Pennsylvania, Philadelphia, PA, United States, [4] Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Milan, Italy, [5] Department of Medical Biotechnologies and Translational Medicine (BioMeTra), University of Milan, Milan, Italy, [6] Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Science (DINOGMI), University of Genoa, Genoa, Italy, [7] UOC Medical Genetics, Giannina Gaslini Institute, Genoa, Italy, [8] Biostatistics Section, Department of Health Science, University of Genoa, Genoa, Italy, [9] Genomics, Development and Disease Section, National Human Genome Research Institute (NHGRI), National Institutes of Health (NIH), Bethesda, MD, United States, [10] Institute of Agriculture Biology and Biotechnology, National Research Council, Pisa, Italy, [11] Department, of Biology, University of Florence, Firenze, Italy

Objectives: Since 2010, several researches demonstrated that microbiota dynamics correlate and can even predispose to Hirschsprung (HSCR) associated enterocolitis (HAEC). This study aims at assessing the structure of the microbiota of HSCR patients in relation to extent of aganglionosis and HAEC status.

Methods: All consecutive HSCR patients admitted to Gaslini Institute (Genova, Italy) between May 2012 and November 2014 were enrolled. Institutional review board (IRB) approval was obtained. Stools were sampled and 16S rDNA V3-V4 regions were sequenced using the Illumina-MiSeq. Taxonomy assignments were performed using QIIME RDP. Alpha diversity indexes were analyzed by Shannon and Simpson Indexes, and Phylogenetic Diversity.

Results: We enrolled 20 patients. Male to female ratio was 4:1. Six patients suffered from Total Colonic Aganglionosis (TCSA). Considering sample site (i.e., extent of aganglionosis), we confirmed the known relationship between sample site and both biodiversity and composition of intestinal microbiota. Patients with TCSA showed lower biodiversity and increased Proteobacteria/Bacteroidetes relative abundance ratio. When addressing biodiversity, composition and dynamics of TCSA patients we could not find any significant relationship with regard to HAEC occurrences.

Conclusions: The composition of HAEC predisposing microbiota is specific to each patient. We could confirm that total colon resections can change the composition of

OPEN ACCESS

Edited by:
Miika Kaleva Arvonen, Kuopio University Hospital, Finland

Reviewed by:
Dipankar Ghosh, Jawaharlal Nehru University, India
Andrea Quagliariello, Bambino Gesù Ospedale Pediatrico, Italy

****Correspondence:***
Alessio Pini Prato
apini@ospedale.al.it
Duccio Cavalieri
duccio.cavalieri@unifi.it;
cavalieri.unifi@gmail.com

Specialty section:
This article was submitted to Pediatric Infectious Diseases, a section of the journal Frontiers in Pediatrics

Received: *31 December 2018*
Accepted: *19 July 2019*
Published: *09 August 2019*

Citation:
Pini Prato A, Bartow-McKenney C, Hudspeth K, Mosconi M, Rossi V, Avanzini S, Faticato MG, Ceccherini I, Lantieri F, Mattioli G, Larson D, Pavan W, De Filippo C, Di Paola M, Mavilio D and Cavalieri D (2019) A Metagenomics Study on Hirschsprung's Disease Associated Enterocolitis: Biodiversity and Gut Microbial Homeostasis Depend on Resection Length and Patient's Clinical History. Front. Pediatr. 7:326. doi: 10.3389/fped.2019.00326

intestinal microbiota and to dramatically reduce microbial diversity. The subsequent reduction of system robustness could expose TCSA patients to environmental microbes that might not be part of the normal microbiota. Future long-term studies should investigate both patients and their family environment, as well as their disease history.

Keywords: metagenomics, enterocolitis, Hirschsprung, *RET* gene, aganglionosis

INTRODUCTION

Enterocolitis (HAEC) is an extremely serious, life-threatening complication that can occur in children with Hirschsprung disease (HSCR) pre- and even post-operatively. Despite a number of studies, the causative agents of HAEC are still elusive. Standard culturomics technologies did not lead to the discovery of microbial pathogens causing HAEC. Novel culture independent approaches based on DNA sequencing of target genes or of the whole bacterial DNA content hold the promise to discover the causative agents and the etiology of HAEC. Since 2006 (1) metagenomics in children has been used to address the role of intestinal microbiota in the etiology of a number of diseases (1–11). Only a minority of papers addressed HSCR and HAEC (6, 12–14). In 2010, our group carried out a pilot genomic study in a single patient with HSCR and demonstrated that HAEC can correlate to changes in gut bacterial dynamics (12). Ward and co-workers later on reported a sustained abnormal microbiota in an animal model of HSCR (13). Yan in 2014 (6) and Frykman in 2015 (14) investigated HSCR patients with or without HAEC. Both authors did not find any significant difference apart from the latter who reported an altered *Candida* community in those with HAEC (14). Of note, Li et al. in 2016 reported a significantly different microbiome in HSCR patients with HAEC (15). Proteobacteria were significantly more represented in patients with HAEC whereas Bacteroidetes were significantly more represented in patients without. Also HSCR patients had a relatively distinct, more stable community than the HAEC and HAEC-R patients (previously settled HAEC episode), suggesting that enterocolitis may either be caused by or result in a disruption of the patient's uniquely adapted intestinal flora. The intestinal microbiota associated with enterocolitis may persist following symptom resolution and can be implicated in symptom recurrence. In another recent study on a mouse model of HSCR, the metagenomics analysis of *Ednrb*$^{-/-}$ and wild type mice showed that mutants had a distinct microbiota with respect to wild type (WT) and that the HAEC group had lower alpha diversity by Chao1 index compared with WT. Also the animals with HAEC had increased proportion of *Akkermansia* genus and reduced Bacteroidetes phylum compared with the NO HAEC and WT groups, suggesting *Akkermansia* may contribute to development of enterocolitis while Bacteroidetes may be protective (16). Finally, another study on Finnish patients showed how those with HD and HAEC had a significantly altered intestinal microbiome compared to healthy individuals, characterized by a lack of richness and pathologic expansions of taxa, particularly Enterobacteria and Bacilli (17). These initial studies did not lead to a predictive profile for HAEC (18, 19). Nonetheless, no mention was done either regarding extent of aganglionosis or diversity measures (15). Here we report results of a metagenomics study on fecal microbiota performed on HSCR patients, addressing limitations, drawbacks and potential benefits of such approach, delineating future perspectives in this field of research.

METHODS

Patients

All pediatric patients with HSCR consecutively admitted to Giannina Gaslini Institute (Genova, Italy) between May 2012 and November 2014 were eligible for this study. Institutional Ethical committee approval was obtained by the Review Board of Giannina Gaslini Institute on November 2009 as part of a wider research project on HSCR. A specific informed consent was signed by all participant families. Inclusion criteria were: (1) diagnosis of HSCR based on histochemical assessment of adequate rectal suction biopsies, as previously reported (20); (2) exhaustive data regarding extent of aganglionosis (adequate intraoperative histology) and regarding personal history with specific regard to previous bouts of HAEC; (3) stool sampling available both from preoperative and postoperative periods. Exclusion criteria were (1) refusal of signing the informed consent; (2) failure to pass internal quality control; (3) inadequate sampling or storage.

Definitions

HSCR, Hirschsprung's disease; RSA, HSCR with aganglionosis extended up to the colonic splenic flexure (i.e., Rectosigmoid Aganglionosis); L-HSCR, Long HSCR with aganglionosis extended beyond the splenic flexure up to ascending colon; TCSA, HSCR with aganglionosis extended to the whole colon (i.e., Total Colonic Aganglionosis); HAEC, Enterocolitis diagnosed according to the combination of Pastor criteria (21) (to confirm the diagnosis) and Elhalaby criteria (22) (to grade HAEC severity).

Stools Collection, Storage, and Delivery

Spontaneous stools were collected and stored frozen (−20°C up to 48 h and −80°C afterwards) until shipment to the reference center for processing of fecal samples (Bethesda, Maryland, USA). Stools from Total Colonic Aganglionosis (TCSA) patients belonged to stoma bags preoperatively and from direct bowel movements postoperatively (in both cases from the ileum) whereas stools from Rectosigmoid Aganglionosis (RSA) or long (L)-HSCR belonged from bowel nursing (enema or rectal tube) preoperatively and from direct bowel movements

postoperatively. Time-points for stool sampling were: (1) before surgery (Timepoint 1, preoperative hospital stay), (2) intraoperative (timepoint 2), and (3) postoperatively 7 to 10 after pull-through (timepoint 3). Only timepoints 1 and 3 (preoperative and postoperative, respectively) were assessed in this study.

Clinical Features and Molecular Genetics

All patients included in this study underwent a thorough phenotype assessment as previously published (23). Demographic data, phenotype results, HAEC status, surgical details, possible complications and long-term outcome were recorded and stored in a digital database according to data protection Act. Furthermore, all patients underwent sequencing of the *RET* gene coding portion (21 exons flanked by at least 20 bp of intronic sequences) as part of the multidisciplinary diagnostic algorithm, as previously published (24). In order to identify mutations that are potentially involved in HSCR, the results of molecular genetics, consisting of (1) putatively pathogenic *RET* mutations; (2) common variants among which those known to represent HSCR risk modulating factors (25), were recorded in the same database as above.

16S rDNA Sequencing and Processing

Amplification and sequencing of the 16S rDNA hypervariable V4 region was performed as previously described using the Illumina MiSeq platform with 150 bp paired end reads and V2 chemistry (26). Sequences were demultiplexed with FLEXBAR (27) and assembled using PEAR (28). Sequences were then quality filtered and restricted to length of 245–255 nt, which retained 9.77 million reads of the filtered 9.78 million reads. The sequences were then analyzed using scripts within the QIIME package (29). Sequences were first clustered into *de novo* operational taxonomic units (OTUs) defined by 97% sequence identity using UCLUST (30). A representative set of sequences for the OTUs was selected using the QIIME "most abundant" selection method. Taxonomy assignments for the representative sequences were performed using the QIIME RDP wrapper with an 80% cut-off for bootstrap confidence in assignment (31). Unclassified sequences and reads classified as Cyanobacteria were removed. The representative sequences were then aligned using PyNAST with a minimum length of alignment cutoff of 150 nucleotides and a minimum percent identity cutoff value of 75% (32). Alignments were performed against the Greengenes 13_8 taxonomy core-set alignment sequences (33). Chimeras were removed from the aligned reads using ChimeraSlayer (34). ChimeraSlayer identified 21,593 OTUs (4.0%) out of 537,481 total OTUs as chimeric. OTUs that possessed <2 sequences and did not occur in more than one sample were removed. Taxonomy summaries were further filtered to only include OTUs that make up at least 0.5% of the total sequences. Rarefaction of the samples was performed on all samples at a depth of 7070 sequences to ensure homogeneity in sample size for downstream analysis. Phylogenetic tree construction of the aligned sequences was performed using FastTree (35). The phylogenetic tree was used for calculation of UniFrac (weighted and unweighted) beta diversity distance matrices (36).

Statistical Analyses

The results were compared according to length of aganglionosis, timepoints, and HAEC status (HAEC episodes either experienced during the study period or before enrollment but reported in personal history (pre-HEAC). The single patient with L-HSCR could not be categorized as classic or ultralong HSCR and was excluded from this statistical analysis in order to avoid misinterpretation (**Table 1**). Diversity indexes as well as phylogenetic analysis were compared in patients with RSA and TCSA regardless of timepoints. TCSA patients that represented the core of our study underwent a further analysis based on HAEC status and timepoints of stool sampling. The R ggplot2 package was used for plotting (37). Alpha diversity index analyses of the samples were conducted using QIIME and the constructed phylogenetic tree to calculate the Shannon index (38), Simpson Index (39), and Phylogenetic Diversity (PD) (40). Pairwise comparison with Wilcoxon rank sum test was used to address Alpha diversity measures. Differences in categorical variables were addressed with chi-square or Fisher exact test, when appropriate. All tests were 2-tailed. A p-value lower than 0.05 was considered as statistically significant.

RESULTS

Overall Samples Distribution

During the study period, we admitted 41 consecutive HSCR patients. Due to the technical difficulties in frozen storage, only 31 of these patients provided adequate material to be sent to Bethesda (NIH, USA) for metagenomics. Of these 31 patients, only 20 provided both preoperative and postoperative specimen that passed internal quality controls. To summarize, out of 41 eligible HSCR patients, preoperative and postoperative stool samples were collected from 20 HSCR patients, 13 suffering from Rectosigmoid Aganglionosis (RSA), 1 L-HSCR and 6 Total Colonic Aganglionosis (TCSA). Median age at enrollment of these 20 patients was 16 months (ranging between 2 months and 13 years). A total of 40 stool samples (20 preoperative and 20 postoperative) were sequenced for 16S rDNA. In order to increase results reliability and to address HSCR forms with the highest risk of HAEC occurrences, we mostly focused on patients with TCSA that represented the core of our study (**Table 1**).

Demographics of TCSA Patients

Six patients with TCSA were included. Male to female ratio was 2:1. Median age at enrollment was 15.5 months (range 9 to 26 months). *RET* mutations were detected in 7 out of 20 patients (35%), 4 of whom suffering from TCSA (67%). Three of these 4 nucleotide changes lead to truncating mutations and, interestingly, none was associated with HSCR cases complicated by HAEC manifestations. Two patients reported a previous history of HAEC (subject A and K), one (subject C) developed HAEC postoperatively well after postoperative timepoint (**Table 1**). *RET* mutations did not correlate either with protection or predisposition to HAEC ($p = 0.4000$). Four associated anomalies were detected in 3 patients. These included Congenital Anomalies of the Kidney and Urinary Tract

TABLE 1 | Patients with TCSA in our series.

	N. of pts	M:F ratio	*RET* mutations (%)	Associated anomalies	HAEC
RSA	13	5.5:1	3 (23%)	4 (31%)	1 (8%)
L-HSCR*	1	n.a.	0 (n.a.)	0 (n.a.)	1 (n.a.)
TCSA	6	2:1	4 (67%)	3 (50%)	3 (50%)
DETAILS ON PATIENTS WITH TCSA (TIME POINTS COMPARED FOR DYNAMIC CHANGES)					
ID of TCSA	**Gender**	**Age**	**Detailed *RET* mutation**	**Associated anomalies**	**HAEC**
A	M	15 months	None	CAKUT	Post severe
C	M	16 months	None	None	Pre severe
K	M	9 months	c.833C>A (p.T278N)	CAKUT	Post severe
L	M	20 months	c.820_820delG (p.A274Rfs*38) c.2075_2076delinsAA (p.A692E)	None	None
N	F	26 months	c.2772_2773insT (D925*)	GUT + EYE	None
Q	F	14 months	c.2829_2830insGGAG (p.I944Gfs*16)	None	None

*The prevalence of associated anomalies is in line to what previously published by our group (20). The table summarizes data regarding RET mutations, Associated Anomalies and HAEC status of the whole series of patients who underwent metagenomics, focusing on patients with TCSA who underwent a deeper assessment. None of the patients received antibiotics close to preoperative timepoint sampling. All TCSA patients received antibiotic prophylaxis for the first 3 days postoperatively (4 to 7 days before postoperative timepoint sampling). N, number; pts, Patients; n.a, not assessed; HAEC, Hirschsprung's associated enterocolitis; RSA, RectoSigmoid Aganglionosis; L-HSCR, Long Hirschsprung form; TCSA, Total Colonic Aganglionosis; RET, Rearranged During Transfection proto-oncogene. Post Severe, Severe HAEC following definitive surgery; Pre Severe, Severe HAEC before diagnosis; CAKUT, Congenital Anomaly of the Kidney and Urinary Tract; GUT, Congenital Intestinal Malformation; EYE, Eye abnormality. *The asterisks in the new nomenclature recommendations indicate the stop codon. In particular it refers to the protein and the stop signal (https://varnomen.hgvs.org/).*

(CAKUT) ($n = 2$), Gastrointestinal anomalies ($n = 1$), and Visual Impairment ($n = 1$) (**Table 1**).

Microbiota Diversity and Composition Comparing TCSA to RSA (20 Patients, 40 Samples)

Diversity

The diversity measures (**Figure 1**) were calculated based on the results derived by the overall assessment of specimen provided by each group (13 RSA and 6 TCSA, both timepoints). The diversity of gut microbial communities was assessed with alpha diversity measures. We calculated the number of observed species-level OTUs in each sample and found that the gut of RSA patients contained a higher number of species-level OTUs than the gut microbiota of TCSA patients (mean difference = 278 species; $p < 0.001$). Similarly, the Shannon index (35), which measures the richness and evenness of a community, was higher in RSA patients than TCSA patients (mean difference = 1.51; $p < 0.01$). Phylogenetic Diversity (PD) (39), which takes into account phylogeny, further confirmed our findings that RSA patients possessed greater microbial diversity compared to TCSA ones (mean difference = 13.42; $p < 0.005$). However, the Simpson Index (38) did not significantly differ between RSA and TCSA patients. The Simpson index measures diversity by calculating the evenness of OTUs in each community and penalizes communities dominated by a small number of OTUs (**Figure 1**).

Composition

We assessed microbial composition by comparing the relative abundances of taxa at the phylum and genus level. At the phylum level differences between RSA and TCSA microbiota were even more striking, specifically with regard to the presence of Bacteroidetes (**Figure 2**). Nearly 70% of RSA patients (69%) had communities composed of over 33% Bacteroidetes whereas all TCSA communities contained <2% Bacteroidetes, which was found to be a significant difference (mean difference = 40.83%; $p < 0.05$). A greater presence of Proteobacteria in TCSA microbiota compared to RSA microbiota was also observed (mean difference = 32.27%; $p < 0.05$). The remaining two classifiable phyla found in all of the samples, Firmicutes and Actinobacteria, did not significantly differ in relative abundance between the two groups.

When considering the communities at the genus level, we observed a prominent composition (>25%) of *Bacteroides* in the majority of the RSA patients' gut microbiota, whereas all of the TCSA patients contained <1% of *Bacteroides* in their gut microbiota. Similarly, we observed a significant increase of *Alistipes* (a genus in the same Phylum as *Bacteroides*, Bacteroidetes) in the gut microbiota of RSA patients when compared to the TCSA patients (mean difference = 1.76%; $p < 0.05$). Conversely, we found that members of the genus *Enterococcus* (a member of the Firmicutes Phylum) were more prevalent in the microbiota of TCSA patients (mean difference = 2.44%; $p < 0.05$).

TCSA Patients (6 Patients, 12 Samples)

Gut microbiota of 6 HSCR patients with TCSA was assessed before and after surgery (comparing timepoints) performed to restore bowel continuity and reverse the stoma (**Figure 3**). All preoperative sampling were obtained by the stoma. Three patients (Subjects L, N, and Q) never experienced

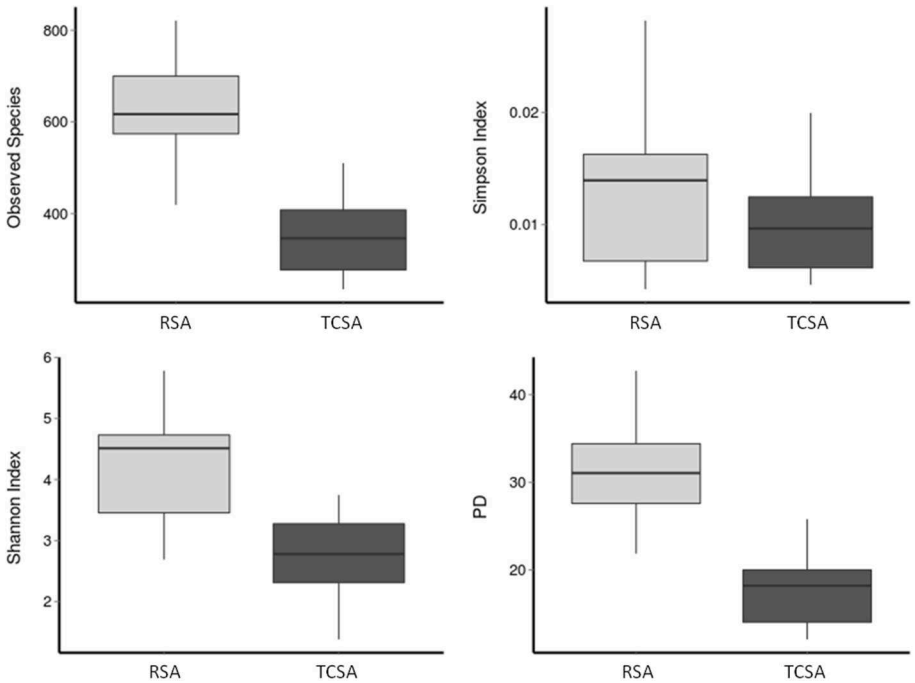

FIGURE 1 | The diversity of gut microbial communities was assessed through four alpha diversity measures. Together, these results suggest that ileal stools belonging to TCSA carry lower microbiota diversity.

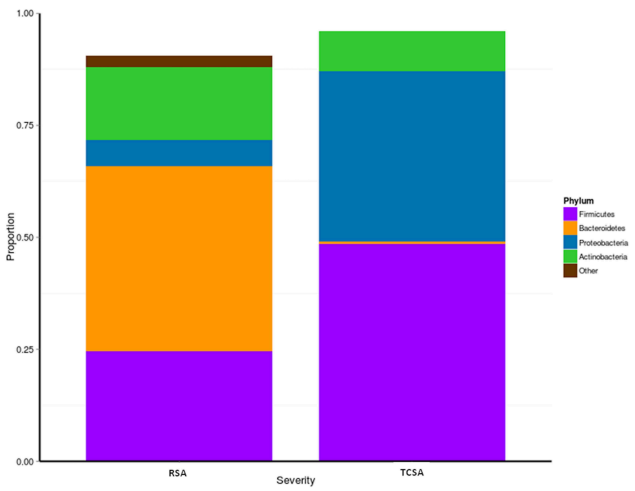

FIGURE 2 | We assessed microbial composition by comparing the relative abundances of taxa at phylum level. When considering the communities at the phylum level, differences between RSA and TCSA microbiota were clearly evident. Bacteroidetes represented over 33% of all bacteria in RSA and where basically absent (<2%) in TCSA. Similarly, Proteobacteria accounted for nearly 40% of all bacteria in TCSA and for <5% in RSA. Conversely, Firmicutes and *Actinobacteria* were present both in RSA and TCSA without statistically significant differences.

episodes of HAEC. One patient (Subject C) experienced an HAEC episode preoperatively, while the two remaining patients experienced post-operative HAEC episodes (Subjects A and K).

We analyzed changes in alpha diversity between the two timepoints using three metrics: OTU richness (the number of observed OTUs in a sample), Shannon Diversity (a measure of OTU richness and evenness), and PD whole tree (a metric that takes phylogeny into account). Four of six subjects increased in Shannon diversity post-operatively. The two subjects that did not increase in Shannon diversity (Subjects A and L) were stable by this metric as well as number of OTUs and PD whole tree. For PD whole tree and OTU richness, most patients also trended toward an increase, though two subjects remained stable or slightly decreased by these metrics (Subject C and Subject N).

Though our sample size was not large enough to adequately power statistical comparisons, we noted that composition of the gut microbiota was highly variable, both pre- and post-operatively, regarding genus level taxonomy. Pre-operatively Subject A was predominantly colonized with *Veillonella*, Subjects K and Q were predominantly colonized by *Enterobacteriaceae* family, Subject C was predominantly colonized with *Clostridium*, and Subject N was predominantly colonized with *Lactobacillus* genus. Subject L was not dominantly colonized by a single genus level taxa and contained a more diverse mixture. For all subjects, there was a drastic shift in composition post-operatively, though we did not detect any specific trend in this regard. At a phylum level, Proteobacteria showed a striking predominance over other phyla either preoperatively or postoperatively but without relationship with HAEC occurrences or HAEC status. In fact, HEAC status, as well as genetic background and phenotype did not show clear correlations with gut microbiota both pre- and post-operatively (Spearman correlation, $p = 0.40$; see **Table 1**, **Figure 3**).

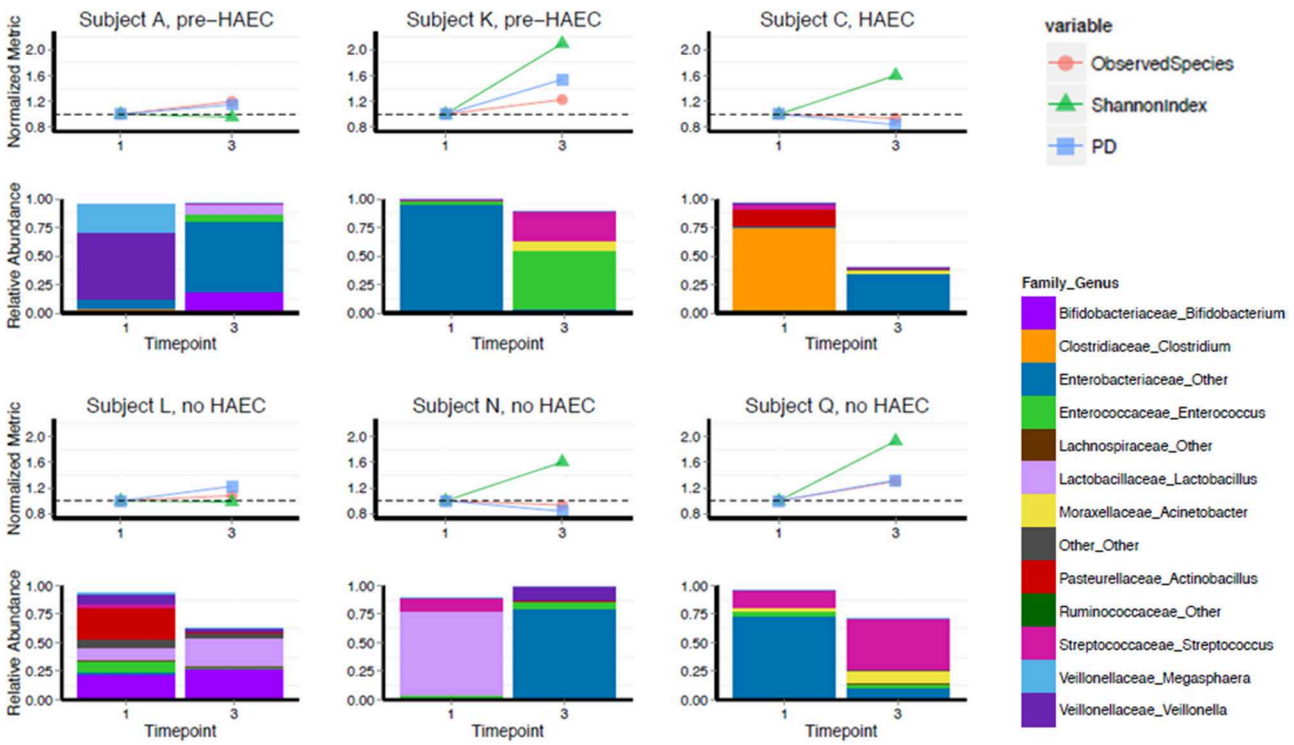

FIGURE 3 | Diversity and composition in TCSA before and after surgery. X-axis depicts timepoints of sample collection: pre-operative ("1") and post-operative ("3"). Subjects A, K, and C either developed HAEC postoperatively (pre-HAEC—HAEC status at stool sampling) or preoperatively (HAEC—HAEC status at stool sampling). Subjects L, N, and Q never developed. The top graph of each panel represents alpha diversity, normalized to values of sample collected pre-operatively, with each color line representing a different metric. The dotted line on each graph is set to 1.0. The bottom panel illustrates relative abundance of taxa at genus level.

DISCUSSION

Our study was aimed at addressing the effect of ultralong aganglionosis (TCSA) and its multiple implications, namely genetic background (higher prevalence of *RET* mutations), motility issues (higher prevalence of obstructive symptoms), and HAEC occurrences [higher frequency of HAEC episodes (12, 21, 22)] on gut microbiota composition. In fact, though it is still unknown if those variables are independent one from the others, RSA and TCSA must be considered separately when addressing HSCR patients.

Only a few published papers compared ileal and colonic fecal microbiota in children with HSCR-associated enterocolitis (HAEC). Available studies mostly refer to newborns or ex-preterm with a history of necrotizing enterocolitis or to children with inflammatory bowel diseases (41–45). The only study comparing microbiota of ileal and colonic stools in healthy subjects, is that by Zoetendal et al. in 2012 (46) but the authors included only adult subjects. Even so, our study confirmed that ileal samples have a lower microbiota diversity when compared to colonic ones, regardless of the presence of a stoma or not, in accordance to work reported by Zoetendal et al. (46). Recently, Barret et al. compared two preterm babies with different stomas (ileal and colonic) and followed them up to 7 months of age (41). They reported that Bifidobacteria and *Enterobacteriaceae* dominate at a genus level in the ileal stools samples (41). Our results are consistent with these findings with similar preponderances at a genus level. Of note, statistical analysis failed to show significant differences in terms of diversity and composition with relation to HAEC occurrences in TCSA patients, thus confirming the heterogeneity of gut microbiota and the difficulty in finding a clear and univocal marker for HAEC predisposition.

Nonetheless, the lower diversity and variable composition of microbiota throughout time points confirms that either with a stoma or not, patients with TCSA have different gut microbiota compared to those with RSA. This aspect could be easily expected basing on previous reports concerning microbiota diversity and composition in different bowel sites (41–46). Even so, as TCSA patients are well known to be susceptible to HAEC (12, 21, 22, 47), we speculate that either reduced diversity in TCSA patients can be merely related to a different sampling site or that this aspect has a pathogenetic role in terms of HAEC predisposition/facilitation. Alternatively, the well-known dysmotility and fecal stasis observed in TCSA patients could lead to bacterial overgrowth that can interfere with homeostasis and bacterial dynamics even more. In case of bacterial overgrowth, a potentially harmful microbial species can outcompete commensals very rapidly and lead to HAEC as a result of systemic reaction to this dysbiosis. This argument is supported by the factthat we could not observe a specific genus or phylum significantly associated with HAEC occurrence within

TCSA patients. Nonetheless, our series of patients with TCSA proved to have a significantly higher abundance of Proteobacteria when compared to RSA ones. In particular, 5 out of 12 samples belonging to TCSA patients were more than 80% composed by Proteobacteria. Conversely, Bacteroidetes were basically absent. The total loss of the colon undermines the existence of a specific organ, composed both of mammalian cells and microbial cells. The colon microbiota provides the system with a fundamental stabilizing function, of crucial importance for the robustness of the whole microbial and immune system. The colonic diversity and richness creates a resilient, reliable, and robust microbial community that can easily cope with potential insults, including the colonization from environmental and food microbiota. In this context, the absence of Bacteroidetes affects the production of short chain fatty acids (SCFAs) which are fundamental for intestinal homeostasis. Our result is thus in agreement with a recent report that showed how fecal samples from HAEC children showed a 4-fold decline in total SCFA concentration vs. non-HAEC HSCR patients (48). In particular, the authors found reduced acetate and increased butyrate in HAEC children, with 10 of 12 butyrate-producing genera as well as 3 of 4 acetate-producing genera demonstrated multi-fold expansion. Yet, we cannot demonstrate whether Proteobacteria preponderance and Bacteroidetes deficiency are linked to sampling site (ileal vs. colonic) or to HAEC predisposition, as recently suggested by Li et al. (15). Noteworthy, sampling site of their HAEC patients belonged more frequently to ileum or right colon, thus introducing a significant bias in the interpretation of their results (15). In TCSA, the ecological resilience of the microbiota to resist an insult is deeply undermined. Thus, TCSA patients have a fragile ileal microbial community, which may be extremely sensitive to dysbiosis that is otherwise harmless in a normal system. This is evident not only by the recurrence of HAEC, but also the variations of microbial compositions between individual patients.

This result is shown not only by the recurrence of HAEC but also from the extreme interindividual variability of the microbial composition. In absence of the colon TCSA patients have a highly variable microbiota, lacking fundamental species associated to heath in normal individuals, such as Bacterioidetes. The abundance of Proteobacteria also reflects the invasion from environmental communities, that are free to thrive in an environment that would be normally precluded to them by the presence of Bacterioidetes. Thus, HAEC should be seen not as a response to a pathogen, but as a response to a community that normally should not be present in the ileum, or in the colon, eliciting deleterious consequences. This explanation of our results is further supported by the most striking finding of a recent study on HAEC in a mouse model of HSCR that suggested how *Akkermansia*, a microorganism normally seen as protective, may contribute to development of enterocolitis while Bacteroidetes may be protective. Less abundant genera that were reduced in HAEC were *Dysgonomonas* and *Clostridium XIVa,* which may play a protective role (16). Taken as a whole our findings suggest that the use of therapies targeted at *Clostridium difficile*, without sufficient confirmation for *Clostridium difficile* overgrowth, might be detrimental in patients with HAEC following total colon resection. Even so, it is still possible that certain composition of the intestinal microbiota, as that reported by Li et al. or in our study, can predispose to HAEC in case of a susceptible genetic and immunologic background well known to be significantly more frequent in TCSA (22). In agreement with previous results (49) 65% of TCSA and 20% of RSA patients in our study have *RET* mutations. As recently published by our group (50), these mutations could determine abnormal expression of RET-dependent and independent pro-inflammatory programs that might predispose to HAEC occurrence. *RET* sequencing in our study could not be correlated to the incidence of HAEC mostly due to the limited number of HAEC-TCSA in our series of patients. We speculate that the interaction of a less diverse and compositionally peculiar gut microbiota (preponderance of Proteobacteria over Bacteroidetes) in patients with an imbalanced RET-dependent and independent immunity (regardless of the loss-of-function effect of *RET* mutations) could facilitate HAEC onset and/or predisposition. In this perspective, the defects observed in HSCR are not restricted to the aganglionic segment but extend to the mucosal immune system within and beyond the gastrointestinal tract, including the microbiota composition (50).

Although our study underlined the potential of metagenomics and improves the understanding of the relationship between microbiota, host, and immune system in patients with HSCR, it suffered important limitations. First of all, although all patients were sampled relatively far away from antibiotic therapy, we cannot exclude long term effects of antibiotic treatment. The microbiota composition proved to be patient-specific and likely depend on patients' personal history, as previously reported by Barrett et al. and Zoetendal et al. (41, 46). In particular, genera and phyla were heterogeneous in patients from our series and there was no specific and reproducible common pattern according to length of aganglionosis, genotype, phenotype, and HAEC status. This was evident when we addressed stools composition of TCSA patients with and without HAEC whose relative abundance of bacterial taxa at each time point were basically not comparable. In context of bacterial diversity and community dynamics, we could observe some of the most intriguing potentials of metagenomics in HSCR, all pointing to a lack of robustness in the gut microbiota. We argue this is due to the loss of the organ principally responsible for maintaining the reservoir of those microbes providing the buffer effect, with a loss of biodiversity corresponding to a loss of resilience. On this specific regard, it appears extremely important to achieve a heathy status by reconstituting this microbial buffer, in order to re-establish a minimal resilience and homeostasis. On the ground of these considerations, strategically designed fecal transplant defined by composition and the abundance enjoy exciting potential to address this issue in TCSA patients.

To conclude, our study confirmed the enormous potential of metagenomics in HSCR but underlined the importance of identifying the proper subset of patients for this powerful methodology. Based on the prevalence of HAEC, patients with TCSA represent the ideal subgroup to study HAEC susceptibility. A longitudinal long-term study on high-risk patients will presumably provide information that could be

better compared, analyzed, and possibly applied or transferred to the general HSCR population. At present, we can only speculate that higher biodiversity could play a role in maintaining gut homeostasis and that its disruption could facilitate HAEC development.

ETHICS STATEMENT

Institutional Ethical committee approval was obtained by the Review Board of Giannina Gaslini Institute on November 2009 as part of a wider research project on HSCR.

AUTHOR CONTRIBUTIONS

AP formulated the idea, designed the study, and wrote and reviewed the manuscript. CB-M performed the DNA extraction and sequencing and analysis. MM coordinated HSCR patients sample acquisition, biobanking, and shipping HSCR patients. VR, IC, SA, and MF sampled stools from HSCR patients and coordinated patients' phenotype and genotype assessment. KH revised the manuscript drafts and supported the lab work. GM revised the manuscript draft and supervised patients data acquisition. FL performed the molecular genetics studies. DL, WP, and DM conceived the study and drafted the manuscript. CD and MD analyzed the microbiome data. DC reviewed the partners contributions, drafted the manuscript, analyzed the microbiome data, and inspired the study.

ACKNOWLEDGMENTS

This work was supported by the Italian Ministry of Health (MOH) Young Researchers Award, code WFR GR-2011-02347381.

SUPPLEMENTARY MATERIAL

Raw data are available at the following link: https://drive.google.com/drive/folders/1MinrLjrlnuufdHRo7aKm1XuvOwNJvR6W.

REFERENCES

1. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*. (2006) 55:205–11. doi: 10.1136/gut.2005.073817
2. Morowitz MJ, Poroyko V, Caplan M, Alverdy J, Liu DC. Redefining the role of intestinal microbes in the pathogenesis of necrotizing enterocolitis. *Pediatrics*. (2010) 125:777–85. doi: 10.1542/peds.2009-3149
3. Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology*. (2011) 141:1782–91. doi: 10.1053/j.gastro.2011.06.072
4. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. *Pediatrics*. (2012) 129:950–60. doi: 10.1542/peds.2011-2736
5. Vallès Y, Gosalbes MJ, de Vries LE, Abellán JJ, Francino MP. Metagenomics and development of the gut microbiota in infants. *Clin Microbiol Infect.* (2012) 18(Suppl. 4):21–6. doi: 10.1111/j.1469-0691.2012.03876.x
6. Yan Z, Poroyko V, Gu S, Zhang Z, Pan L, Wang J, et al. Characterization of the intestinal microbiome of Hirschsprung's disease with and without enterocolitis. *Biochem Biophys Res Commun.* (2014) 445:269–74. doi: 10.1016/j.bbrc.2014.01.104
7. Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. *J Gastroenterol Hepatol.* (2015) 30:51–8. doi: 10.1111/jgh.12727
8. Stewart CJ, Nelson A, Scribbins D, Marrs EC, Lanyon C, Perry JD, et al. Bacterial and fungal viability in the preterm gut: NEC and sepsis. *Arch Dis Child Fetal Neonatal Ed.* (2013) 98:F298–303. doi: 10.1136/archdischild-2012-302119
9. Romano-Keeler J, Moore DJ, Wang C, Brucker RM, Fonnesbeck C, Slaughter JC, et al. Early life establishment of site-specific microbial communities in the gut. *Gut Microbes.* (2014) 5:192–201. doi: 10.4161/gmic.28442
10. Tjellström B, Högberg L, Stenhammar L, Magnusson KE, Midtvedt T, Norin E, et al. Effect of exclusive enteral nutrition on gut microflora function in children with Crohn's disease. *Scand J Gastroenterol.* (2012) 47:1454–9. doi: 10.3109/00365521.2012.703234
11. Aomatsu T, Imaeda H, Fujimoto T, Takahashi K, Yoden A, Tamai H, et al. Terminal restriction fragment length polymorphism analysis of the gut microbiota profiles of pediatric patients with inflammatory bowel disease. *Digestion*. (2012) 86:129–35. doi: 10.1159/000339777
12. De Filippo C, Pini-Prato A, Mattioli G, Avanzini S, Rapuzzi G, Cavalieri D, et al. Genomics approach to the analysis of bacterial communities dynamics in Hirschsprung's disease-associated enterocolitis: a pilot study. *Pediatr Surg Int.* (2010) 26:465–71. doi: 10.1007/s00383-010-2586-5
13. Ward NL, Pieretti A, Dowd SE, Cox SB, Goldstein AM. Intestinal aganglionosis is associated with early and sustained disruption of the colonic microbiome. *Neurogastroenterol Motil.* (2012) 24:874–e400. doi: 10.1111/j.1365-2982.2012.01937.x
14. Frykman PK, Nordenskjöld A, Kawaguchi A, Hui TT, Granström AL, Cheng Z, et al. Characterization of bacterial and fungal microbiome in children with Hirschsprung disease with and without a history of enterocolitis: a multicenter study. *PLoS ONE*. (2015) 10:e0124172. doi: 10.1371/journal.pone.0124172
15. Li Y, Poroyko V, Yan Z, Pan L, Feng Y, Zhao P, et al. Characterization of intestinal microbiomes of Hirschsprung's disease patients with or without enterocolitis using illumina-MiSeq high-throughput sequencing. *PLoS ONE*. (2016) 11:e0162079. doi: 10.1371/journal.pone.0162079
16. Cheng Z, Zhao L, Dhall D, Ruegger PM, Borneman J, Frykman PK. Bacterial microbiome dynamics in post pull-through Hirschsprung-Associated Enterocolitis (HAEC): an experimental study employing the endothelin receptor B-null mouse model. *Front. Surg.* (2018) 5:30. doi: 10.3389/fsurg.2018.00030
17. Neuvonen MI, Korpela K, Kyrklund K, Salonen A, de Vos W, Rintala RJ, et al. Intestinal microbiota in Hirschsprung disease. *J Pediatr Gastroenterol Nutr.* (2018). 67:594-600. doi: 10.1097/MPG.0000000000001999
18. Jiao CL, Chen XY, Feng JX. Novel insights into the pathogenesis of Hirschsprung's-associated enterocolitis. *Chin Med J.* (2016) 129:1491–7. doi: 10.4103/0366-6999.183433
19. Gosain A, Brinkman AS. Hirschsprung's associated enterocolitis. *Curr Opin Pediatr.* (2015) 27:364–9. doi: 10.1097/MOP.0000000000000210
20. Martucciello G, Pini Prato A, Puri P, Holschneider AM, Meier-Ruge W, Jasonni V, et al. Controversies concerning diagnostic guidelines for anomalies of the enteric nervous system: a report from the fourth International Symposium on Hirschsprung's disease and related neurocristopathies. *J Pediatr Surg.* (2005) 40:1527–31. doi: 10.1016/j.jpedsurg.2005.07.053
21. Pastor AC, Osman F, Teitelbaum DH, Caty MG, Langer JC. Development of a standardized definition for Hirschsprung's-associated enterocolitis: a Delphi analysis. *J Pediatr Surg.* (2009) 44:251–6. doi: 10.1016/j.jpedsurg.2008.10.052
22. Elhalaby EA, Coran AG, Blane CE, Hirschl RB, Teitelbaum DH. Enterocolitis associated with Hirschsprung's disease: a clinical-radiological characterization based on 168 patients. *J Pediatr Surg.* (1995) 30:76–83. doi: 10.1016/0022-3468(95)90615-0

23. Pini Prato A, Rossi V, Mosconi M, Holm C, Lantieri F, Griseri P, et al. A prospective observational study of associated anomalies in Hirschsprung's disease. *Orphanet J Rare Dis.* (2013) 8:184. doi: 10.1186/1750-1172-8-184
24. Pini Prato A, Musso M, Ceccherini I, Mattioli G, Giunta C, Ghiggeri GM, et al. Hirschsprung disease and congenital anomalies of the kidney and urinary tract (CAKUT): a novel syndromic association. *Medicine.* (2009) 88:83–90. doi: 10.1097/MD.0b013e31819cf5da
25. Emison ES, Garcia-Barcelo M, Grice EA, Lantieri F, Amiel J, Burzynski G, et al. Differential contributions of rare and common, coding and noncoding *Ret* mutations to multifactorial Hirschsprung disease liability. *Am J Hum Genet.* (2010) 87:60–74. doi: 10.1016/j.ajhg.2010.06.007
26. Hannigan GD, Hodkinson BP, McGinnis K, Tyldsley AS, Anari JB, Horan AD, et al. Culture-independent pilot study of microbiota colonizing open fractures and association with severity, mechanism, location, and complication from presentation to early outpatient follow-up. *J Orthopaed Res.* (2014) 32:597–605. doi: 10.1002/jor.22578
27. Dodt M, Roehr J, Ahmed R, Dieterich C. FLEXBAR—flexible barcode and adapter processing for next-generation sequencing platforms. *Biology.* (2012) 1:895–905. doi: 10.3390/biology1030895
28. Zhang J, Kobert K, Flouri T, Stamatakis A. PEAR: a fast and accurate illumina paired-end reAd mergeR. *Bioinformatics.* (2014) 30:614–20. doi: 10.1093/bioinformatics/btt593
29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* (2010) 7:335–6. doi: 10.1038/nmeth.f.303
30. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* (2010) 26:2460–1. doi: 10.1093/bioinformatics/btq461
31. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microb.* (2007) 73:5261–7. doi: 10.1128/AEM.00062-07
32. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics.* (2010) 26:266–7. doi: 10.1093/bioinformatics/btp636
33. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microb.* (2006) 72:5069–72. doi: 10.1128/AEM.03006-05
34. Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G., et al. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* (2011) 21:494–504. doi: 10.1101/gr.112730.110
35. Price MN, Dehal PS, Arkin AP. FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS ONE.* (2010) 5:e9490. doi: 10.1371/journal.pone.0009490
36. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol.* (2005) 71:8228–35. doi: 10.1128/AEM.71.12.8228-8235.2005
37. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag (2009). doi: 10.1007/978-0-387-98141-3
38. Shannon CE. The mathematical theory of communication. 1963. *MD Comput Comput Med Pract.* (1996) 14:306–17.
39. Simpson EH. Measurement of diversity. *Nature.* (1949) 163:688. doi: 10.1038/163688a0
40. Faith DP. Conservation evaluation and phylogenetic diversity. *Biol Conserv.* (1992) 61:1–10. doi: 10.1016/0006-3207(92)91201-3
41. Barrett E, Guinane CM, Ryan CA, Dempsey EM, Murphy BP, O'Toole PW, et al. Microbiota diversity and stability of the preterm neonatal ileum and colon of two infants. *Microbiologyopen.* (2013) 2:215–25. doi: 10.1002/mbo3.64
42. Wall R, Hussey SG, Ryan CA, O'Neill M, Fitzgerald G, Stanton C, et al. Presence of two Lactobacillus and Bifidobacterium probiotic strains in the neonatal ileum. *ISME J.* (2008) 2:83–91. doi: 10.1038/ismej.2007.69
43. Haberman Y, Tickle TL, Dexheimer PJ, Kim MO, Tang D, Karns R, et al. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *J Clin Invest.* (2014) 124:3617–33. doi: 10.1172/JCI75436
44. Wagner J, Maksimovic J, Farries G, Sim WH, Bishop RF, Cameron DJ, et al. Bacteriophages in gut samples from pediatric Crohn's disease patients: metagenomic analysis using 454 pyrosequencing. *Inflamm Bowel Dis.* (2013) 19:1598–608. doi: 10.1097/MIB.0b013e318292477c
45. Conte MP, Schippa S, Zamboni I, Penta M, Chiarini F, Seganti L, et al. Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease. *Gut.* (2006) 55:1760–7. doi: 10.1136/gut.2005.078824
46. Zoetendal EG, Raes J, van den Bogert B, Arumugam M, Booijink CC, Troost FJ, et al. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J.* (2012) 6:1415–26. doi: 10.1038/ismej.2011.212
47. Menezes M, Pini Prato A, Jasonni V, Puri P. Long-term clinical outcome in patients with total colonic aganglionosis: a 31-year review. *J Pediatr Surg.* (2008) 43:1696–9. doi: 10.1016/j.jpedsurg.2008.01.072
48. Demehri FR, Frykman PK, Cheng Z, Ruan C, Wester T, Nordenskjöld A, et al. Altered fecal short chain fatty acid composition in children with a history of Hirschsprung-associated enterocolitis. *J Pediatr Surg.* (2016) 51:81–6. doi: 10.1016/j.jpedsurg.2015.10.012
49. Lantieri F, Griseri P, Amiel J, et al. The molecular genetics of Hirschsprung's disease. In: Holschneider AM, Puri P, editors. *Hirschsprung's Disease and Allied Disorders*. Berlin: Springer-Verlag (2008). p. 63–78. doi: 10.1007/978-3-540-33935-9_5
50. Rusmini M, Griseri P, Lantieri F, Matera I, Hudspeth KL, Roberto A, et al. Induction of RET dependent and independent pro-inflammatory programs in human peripheral blood mononuclear cells from Hirschsprung patients. *PLoS ONE.* (2013) 8:e59066. doi: 10.1371/annotation/d3a96ff5-2a66-4454-8d8d-932ad4cfe906

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

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership