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Multisystem inflammatory syndrome in children

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Multisystem inflammatory syndrome in children

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Editorial: Multisystem inflammatory syndrome in children

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Editorial on the Research Topic
Multisystem inflammatory syndrome in children

Multisystem inflammatory syndrome is a severe complication associated with COVID-19, initially recognized as a distinct clinical entity in 2020 (1). In a bulletin from the UK's National Health Service (NHS) in late April 2020, highlighting a new multi-system inflammatory condition involving a small number of children, the term "PIMS" (Pediatric Inflammatory Multisystem Syndrome associated with SARS-CoV-2) was initially used. Primarily observed in pediatric patients, it was subsequently classified by the Centers for Disease Control and Prevention as Multisystem Inflammatory Syndrome in Children (MIS-C) (2). MIS-C manifests as a rare delayed hyperinflammatory response following SARS-CoV-2 infection. Although the exact pathophysiology remains unclear, the SARS-CoV-2 coronavirus seems to trigger a dysregulated pathological immune response in the host, leading to systemic vasculitis and widespread acute organ damage (3).

Like the viral infection caused by COVID-19, also other infectious agents, such as the Epstein-Barr virus (EBV), have also provided the opportunity to analyze the complex mechanisms leading to hyperinflammatory states. EBV can influence the expression and modulation of TLR7 and TLR9 signaling pathways, and consequently the transcription factor NF-κB activation (Tan et al.) (4). Liu et al. observed how the type of interplay between EBV and TLRs defines the disease outcome. Patients with chronic active EBV (CAEBV) showed a sustained and heightened activation of TLR7 and TLR9 along with their downstream signaling mediators (Liu et al.). This could suggest how a deficit in the self-regulation of negative feedback mechanisms associated with TLRs predisposes to a prolonged, and excessive inflammatory response, potentially contributing to an unfavorable outcome.

MIS-C presents with a heterogeneous clinical profile and variable severity, involving multiple organs and characterized by a state of hyperinflammation, often requiring intensive care. It shares clinical similarities with Kawasaki Disease (KD)-like shock syndrome, explaining how treatment protocols have been mostly derived from those used in KD (5).

About cardiac manifestations of these diseases, myocarditis and left ventricular systolic dysfunction are very common in MIS-C patients and rare in KD. Regarding the occurrence

TABLE 1 Difference between multisystem inflammatory syndrome in children and Kawasaki disease.

	MIS-C	KD
Age	8–11 years	Infants and children <5 years of age
Sex ratio (M/F)	1:1	1.5:1
Gastrointestinal symtoms	+	−
Myocardial dysfunction	+	−
Coronary Aneurism	+	−
Organ dysfunction	+	−
Inflammatory markers	+	+
Fatality rate	1.4%–1.7%	0.01%

coronary aneurysm (CAA), a typical complication of KD, after the introduction of intravenous immunoglobulin treatment, they can be seen only in 4% of the patients, while in MIS-C, 14%–36% exhibit CAA (6).

Difference between MIS-C and KD has shown in Table 1.

This new condition has attracted significant scientific interest and has resulted in numerous publications (7–12), (Table 2). However, despite advancements, many aspects of MIS-C, including epidemiology, pathogenesis, clinical spectrum, and long-term outcomes, still remain poorly understood, providing numerous avenues for future research. Furthermore, although it has become increasingly clear that MIS-C and KD exhibit significant differences, some intriguing points of intersection seem to exist in their pathogenetic mechanisms that still need to be defined (13).

At the beginning of the pandemic, differentiating children with acute severe COVID-19 infection from those who developed the post-infectious hyperinflammatory syndrome, MIS-C, proved challenging (14). In this regard, Jiju et al. in their retrospective study compared the features of 161 symptomatic acute COVID-19 and 50 MIS-C patients (≤19 years) admitted to a tertiary pediatric hospital in the North-West of England (Jiju et al.). They observed that MIS-C patients were older, with a median of 10.3 years, developed the disease later with respect to the primary infection, and often had associated comorbidities. Clinically, they typically presented with abdominal and neurological symptoms, higher inflammatory markers, and showed a more severe disease course, with a higher incidence of death.

European countries and the United States mostly contributed to the majority of scientific publications regarding MIS-C at the onset of the pandemic (15, 16). Conversely, MIS-C has rarely been reported in Chinese children. The reasons could be due to differences in prevalence rates of infection in children and differences in ethnic, genetic background, and SARS-CoV-2 subtypes (17). Wang et al. described a 4-year-old Chinese girl with severe COVID-19 infection complicated with MIS-C successfully treated according to an expert consensus statement Wang et al. (18).

MIS-C may present with a variety of clinical presentations, also in terms of severity. Efforts have been made to comprehend the role of biomarkers in predicting the disease course and outcome (19, 20). For example, older age and initial serum albumin levels have been identified as early indicators, helping to recognize children at high risk for intensive care unit admission (21).

Snipaitiene et al. in a retrospective study involving 43 patients evaluated the role of platelet (PLT) count and PLT indices (plateletcrit, mean platelet volume, and platelet distribution width) in predicting MIS-C severity in children who presented at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos. They found that these markers allowed better prediction of MIS-C severity (Snipaitiene et al.). With the same purpose, Fastiggi et al. analyzed the role of the thyroid axis, Euthyroid Sick Syndrome (ESS), in a single-center observational study involving 42 patients with MIS-C, showing it to be a potential predictor of severe MIS-C course (Fastiggi et al.).

The first-line treatment for MIS-C, usually based on intravenous immunoglobulin (IVIG) and corticosteroids, aim to address the inflammatory response and symptoms associated with this condition (22).

In some cases, additional therapies may be considered based on the severity of the condition and individual patient needs. Among

TABLE 2 Top 6 articles related to multisystem inflammatory syndrome, MIS-C, updated as of January 29, 2019.

Year	Title	First author	Journal	References	NIH percentile	RCR
2021	American college of rheumatology clinical guidance for multisystem inflammatory syndrome in children associated with SARS-CoV-2 and hyperinflammation in pediatric COVID-19: version 3	Henderson LA	Arthritis Rheumatol.	(7)	99.8	31.97
2020	Clinical characteristics of children and young people admitted to hospital with COVID-19 in united kingdom: prospective multicentre observational cohort study	Swann OV	BMJ	(8)	99.7	29.18
2020	Multisystem inflammatory syndrome in children associated with severe acute respiratory syndrome coronavirus 2 infection (mis-c): a multi-institutional study from New York City	Kaushik S	J Pediatr.	(9)	99.6	23.26
2020	American college of rheumatology clinical guidance for multisystem inflammatory syndrome in children associated with SARS-CoV-2 and hyperinflammation in pediatric COVID-19: version 1	Henderson LA	Arthritis Rheumatol.	(10)	99.6	22.40
2021	Association of intravenous immunoglobulins plus methylprednisolone vs. immunoglobulins alone with course of fever in multisystem inflammatory syndrome in children	Ouldali N	JAMA	(11)	99.5	21.13
2020	Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2	Diorio C	J Clin Invest.	(12)	99.4	19.09

RCR, relative citation ratio.

the treatments included in the expert consensus statement anakinra has been extensively used in this condition (23). However, data on the efficacy and safety of anakinra in patients with MIS-C are still lacking (16). Licciardi et al. in their retrospective multicenter study compared patients treated with anakinra in the ICU with those treated in the pediatric wards, observing that anakinra resulted to be efficacious and safe (Licciardi et al.).

In a MIS-C patients resistant to first line therapy, Tocilizumab, anti-IL 6, has been studied as a second line of treatment. In fact Çelikel et al. investigated the efficacy of anakinra, and/or tocilizumab in resistant patients with severe MIS-C admitted in the PICU. They enrolled at 33 patients with MIS-C with a median age of 9 years. All the patients were given first line of therapy. 23/33 (69.9) patients took Anakinra. Two patients were switched to tocilizumab because they were unresponsive to anakinra. All patients showed an increase in lymphocyte and platelet counts and a decrease in ferritin, B-type natriuretic peptide, and troponin levels after first week of treatment (24).

Moreover, Niño-Taravilla et al. describe a case of 8-year-old boy with severe MIS-C treated with tocilizumab (8 mg/kg) and corticosteroid therapy. Two days after the start of treatment, he showed an improvement in symptoms, cardiac function and laboratory tests (25).

Intravenous immunoglobulins (IVIG) is the well-established primary therapeutic option for KD and has also been extensively used in MIS-C (18). However, IVIG may entail rare, often under-recognized side effects such as headaches, hyperviscosity, and hemolysis. The passive transfer of isoagglutinins, typically anti-A or anti-B antibodies, has been recognized as the main causative factor for IVIG-associated hemolytic anemia (26).

In the context of KD, IVIG-associated hemolysis affects up to 16% of the patients. Sedlin et al. reported the first two pediatric patients, a 2 and an 8-year-old girl, diagnosed with MIS-C developing this adverse effect after IVIG therapy (27).

Evidence regarding second-line therapy in patients with resistant KD remains contradictory, a second infusion of IVIG still represents one of the most frequently employed options in such cases (Sedlin et al.). However, given the side effects associated with high IVIG doses and the availability of alternative treatments, several studies have explored their efficacy and side effects (28). Pan et al. conducted a network meta-analysis and used an aggregate Data Drug Information System software v.1.16.5 incorporating clinical trials comparing the safety and efficacy of infliximab, second IVIG infusions, and intravenous pulse methylprednisolone (IVMP) Pan et al. (29). Infliximab emerged as the optimal second-line treatment choice, although associated with an increased susceptibility to hepatomegaly. No significant differences in the risk of developing a coronary artery aneurysm among these three different options emerged.

KD and MIS-C are now recognized under the same hyperinflammatory umbrella, yet they are considered distinct diseases. However, during the early stages of the pandemic, the emergence of a new condition with a KD-like presentation posed diagnostic challenges. Initially, the boundaries between MIS-C and KD seemed blurred, sparking renewed interest among clinicians and scientists in the study of KD (30). Tan and colleagues in their bibliometric analysis have explored the current hotspots and trends in research, founding that in the period 2017–2021, more than 5,500 articles on KD have been published in the Web of Science and Scopus databases, predominantly authored by researchers from Japan, the USA, and China, with a specific focus on "COVID-19" and "multisystem inflammatory disease" (Pan et al.).

In conclusion, despite the profound impact of the COVID-19 pandemic, it has nonetheless left a significant mark on the scientific landscape. This is evident in the collaborative research endeavors that undeniably contributed to advancements in medical knowledge.

Author contributions

AM: Writing – original draft, Writing – review & editing. TG: Writing – original draft, Writing – review & editing.

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.

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Multisystem inflammatory syndrome (MIS-C) with SARS-CoV-2 omicron variant BA.2.38 in a four-year-old Chinese girl: A case report

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We report a severe COVID-19 complicated with MIS-C in a girl treated by the author in China, and discuss the current research status and progress in the diagnosis and therapy of MIS-C in children. The patient was a 4-year-old child previously healthy who was referred to the hospital with a complaint of fever, finally, Multisystem inflammatory syndrome was diagnosed with COVID-19.

KEYWORDS

SARS-CoV-2, multisystem inflammatory syndrome, MIS-C, COVID-19, children

Since the outbreak of coronavirus disease 2019 (COVID-19), severe cases in children in China have been significantly lower than in adults. In particular, Multisystem inflammatory syndrome in children (MIS-C) is scarcely reported in China. In late April 2020, clinicians in the United Kingdom reported a cluster of eight previously healthy children presenting with hyperinflammatory shock during the COVID-19 pandemic, showing features similar to atypical Kawasaki disease (KD), Kawasaki disease shock syndrome, or toxic shock syndrome (1). The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) named the disease multisystem inflammatory syndrome in children (MIS-C). Since the first case was reported, there have been additional similar reports from other countries, primarily in United States, Italy, United Kingdom, France, and Switzerland, and these regions have experienced a considerable number of cases of MIS-C during the SARS-CoV-2 pandemic (1–5). MIS-C has rarely been reported occurring in China in published reports, although COVID-19 was first reported in Wuhan, the worst affected city by SARS-CoV-2 in China. Reasons for this observation are unclear, may involve differences in prevalence rates of infection in children, the infection rate and fatality rate were much lower in China than that of the main European and American epidemic cities; In addition, the differences in ethnic or genetic background and SARS-CoV-2 subtypes, host factors, early large-scale screening and early treatment with immunomodulators may also involve (6). In this article, we report an MIS-C in a girl in China and discuss the current and new progress in the diagnosis and treatment of MIS-C in children based on the literature.

Case presentation

A 4-year-old female presented to the hospital with a fever for 5 days and was admitted on July 31, 2022. COVID-19 was confirmed by the detection of two SARS-CoV-2 viruses using a nucleic acid test (throat swabs), and the patient was transferred to a designated hospital for treatment. She had a paroxysmal dry cough without obvious dyspnea and continuous fever of up to 41 °C, but without chills or convulsions. She had no gastrointestinal symptoms such as nausea, vomiting, or diarrhea, but with bad appetite. She was previously healthy. Her peripheral oxygen saturation (SpO2) was 78% and SpO2 was 95% under nasal tube oxygen (2 L/min). Temperature(T): 39. 2 °C; pulse (P): 94 times/min; respiration(R): 20 times/min; capillary refill time (CRT): 3s. She had poor mental health, cyanosis of the lips and fingernails, low temperature of the extremities, the flowering of the skin during high fever, congestive rash scattered throughout the body, mild bulbar conjunctival congestion, chapped lips, and a strawberry tongue. Lymph nodes with a diameter of 2 cm were palpable in her right neck, with a range of motion and no tenderness. The liver was palpable 3 cm below the ribs with clear margins, and the spleen below the ribs was not palpable. Laboratory biomarkers (Table 1) indicating inflammation: an elevated white blood cell, erythrocyte sedimentation rate, C-reactive protein level, procalcitonin,interleukin-6; Serum amyloid A, lymphocytopenia, neutrophilia, elevated ferritin level, lactic dehydrogenase, creatine kinase, hypoalbuminemia, and an elevated d-dimer level and fibrinogen level. brain natriuretic peptide (BNP)100pg/ml; troponin 0.004ng/ml.Chest CT showed a few inflammatory lesions in the posterior segment of the right upper lobe, the medial segment of the middle lobe, and the basal segment of the lower lobe of both lungs (Figure 1). Abdominal CT an enlarged liver volume, and echocardiogram demonstrated tricuspid regurgitation rate was 223 cm/s, left ventricular ejection fraction was 65%, and the inner diameters of each heart cavity and great vessels were normal. According to the diagnostic criteria of Diagnosis, treatment, and prevention of severe acute respiratory syndrome coronavirus 2 infections in children: expert' consensus statement (Fourth Edition) (7), She was diagnosed with COVID-19 (severe) with the MIS-C. In our case, the diagnosis was based on the following: (1) serious illness leading to hospitalization; (2) a 4-year-old child with evidence of COVID-19 infection; (3) persistent high fever that lasted for more than 5 days; (4) multisystem organ involvement: respiratory symptoms such as paroxysmal cough; acute gastrointestinal problems such as vomiting and bad appetite; conjunctival hyperemia of both eyes; systemic pleomorphic derma; headache; abnormal coagulation function; (5) evidence of the abnormal elevation of laboratory inflammatory markers that could not be explained by other pathogenic microorganisms.

According to Diagnosis, treatment and prevention of severe acute respiratory syndrome coronavirus 2 infection in children: expert' consensus statement (Fourth Edition) (7), the therapy included intravenous immunoglobulin 1 g/kg, once a day for 2 consecutive days, methylprednisolone 1 mg/kg, twice a day for 4 consecutive days, low molecular weight heparin calcium 100 IU/kg subcutaneous injection, twice a day, oral enteric aspirin 5 mg/kg, cough medicine and symptomatic and supportive treatment. On the 4th day, the child developed vomiting and headache for 2 days. On the 6th day after treatment, the patient's temperature gradually returned to normal, and the pleomorphic rash on her face and trunk gradually converged into flakes (Figure 2). The cough disappeared on the 9th day, and the general condition also improved. Most importantly, her SARS-CoV-2 viral nucleic acid test also turned negative on the 9th day, and other laboratory biomarkers indicating inflammation were back to normal. The children were followed up for 2-3 weeks after discharge by gradually reducing the dose of oral methylprednisolone. The specific laboratory results for the children are presented in Table 1. Day 1 is the first day of admission.

Discussion

We report a previously healthy child who was diagnosed with MIS-C and the clinical manifestations are similar to those of KD, who was improved with intravenous immunoglobulin and systemic glucocorticoids, oral enteric aspirin, subcutaneous injection of low molecular weight heparin based on the elevated d-dimer level.

Severe children cases with COVID-19 are more prevalent in children under 1 year of age, in particular those who utilize immunosuppression, and those with underlying illnesses, and most of them are accompanied by respiratory symptoms, whereas the symptoms are milder than those observed in adults (8). Fever is the main symptom in 93.3% in China, with an average duration of 2–3 days, and most of them have a favorable prognosis. MIS-C is defined as clinically severe illness requiring hospitalization with fever, inflammatory marker elevation, and multisystem organ dysfunction in the setting of recent proven or probable SARS–CoV-2 infection, and in the absence of an alternative likely explanation (9). MIS-C is a severe inflammatory syndrome diagnosed as KD manifested by toxic shock and cardiogenic or vascular paralytic shock. Some children are directly or indirectly related to SARS-CoV-2. It has been reported that MIS-C predominantly occurs in previously healthy children and adolescents (10), in which they are mainly systemic multisystem damage. Patients with MIS-C were noted to have a high frequency of fever and gastrointestinal symptoms including abdominal pain, vomiting, and diarrhea. Cough and respiratory distress were reported not very common

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TABLE 1 Laboratory indexes.

Measure	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Reference ranges
Complete blood count							
WBC($\times 10^9$/L)	12.2	5.5	4.4	5.3	7.4	5.8	4.4–11.9
HGB (g/L)	119	119	127	119	116	115	112–149
PLT ($\times 10^9$/L)	224	209	237	195	190	219	100–300
NEU (%)	88	83	61	77	59	67	22–65
LYM (%)	8.6	14.5	32.7	20.2	32.5	26.9	23–69
MON (%)	3	2	6	3	8	6	2–21
Inflammatory markers							
PCT (ng/ml)	5.941	5.242	2.611	1.706	16.84	0.143	0–0.5
CRP (mg/L)	90.08	94.73	58.69	23.65	12.39	7.05	0–10
IL-6 (pg/ml)	96.19	16.9	6.03	3.7	0.59	5.49	1.18–5.30
SAA (ug/ml)	243.6	68.41	41.48	10.09	10.18	21.61	0–10
ESR (mm/h)	-	27	34	-	45	17	0–20
FER (ng/ml)	-	-	170.8	-	-	240.5	25–200
Coagulopathy							
PT (s)	13.5	12.1	10.8	9.6	9.5	9.3	9.8–12.1
APTT (s)	38.2	30.6	32	30.4	30.9	26.9	23.3–31.3
INR	1.15	1.03	0.91	0.81	0.8	0.78	0.8–1.5
D-D (ug/ml)	6.89	4.61	1.43	0.82	0.89	1.00	0–0.55
FIB (g/L)	3.64	3.41	3.2	2.24	2.21	2.27	1.8–3.5
Nucleic acid test results (Omicron BA.2.38)							
ORF1abgene	34	negative	32	-	negative	negative	negative
N gene	34	37	31	-	negative	negative	negative
IgMDL (S/CO)	-	1.01	-	-	-	1.2	negative
IgGDL (S/CO)	-	29.86	-	-	-	123.65	negative
Biochemical indicators							
ALB (g/L)	35.8	33.8	34.5	31.7	31.9	33	39–54
GLO (g/L)	21.4	20.7	35.9	42	37.1	49.7	15–34
ALT (U/L)	10	26	24	20	21	24	7–30
AST (U/L)	37	63	43	34	39	31	14–44
TB (umol/L)	13.7	9.9	7.4	5	5.4	3.3	0.14–9.66
GLU (mmol/L)	4.34	3.78	6.6	6.71	4.25	5.7	3.3–5.6
Na+ (mmol/L)	133	133	134.6	134.9	138.4	138.9	135–145
K+ (mmol/L)	3.46	4.46	4.07	3.9	3.36	4.28	3.7–5.2
Ca2+ (mmol/L)	2.1	2.15	2.17	2.14	2.14	2.34	2.5–3.0
LDH (U/L)	448	511	510	415	405	333	120–250
CK (U/L)	149	137	352	283	195	43	40–200
CK-MB (U/L)	16	15	27	44	49	30	0–25
CREA (umol/L)	28	212	27	25	27	22	19–44
UREA(mmol/L)	3.2	5.1	2.8	2.5	2.6	2	2.5–6.5
TG(mmol/L)	0.75	1.61	1.29	1.31	1.47	1.06	0–1.6

"-" indicates undetermined.WBC, white blood cell; HGB, hemoglobin; PLT, platelet count;NEU, Neutrophil; LYM, lymphocytes; PCT, procalcitonin;CRP, C-reactive protein; IL-6, interleukin-6; SAA, Serum amyloid A; ESR, erythrocyte sedimentation rate; FER, ferritin; PT, prothrombin time;APTT, activated partial thromboplastin time; INR, international normalized ratio; D-D, D-dimer;FIB, fibrinogen; ALB, serum albumin; GLO, seroglobulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bile acid; GLU, glucose; LDH, lactic dehydrogenase; CK, creatine kinase;CREA, Creatinine; UREA, urea nitrogen; TG, triglyceride.

or not severe (11). Blood parameters showed neutrophilia in 83% of cases and a high CRP in 94%. However, only 41% demonstrated pulmonary changes on chest imaging. MIS-C is a serious disease that can progress rapidly and worsen in a short

time. The severity of illness was high with 68% of cases requiring intensive care admission, 63% requiring inotropic support, 28% of cases needing some form of respiratory support (12), and approximately 60% of patients that were diagnosed with MIS-C presented with shock (11). Studies have reported that the fatality rate of MIS-C in the United Kingdom and the United States can reach 2% (4, 5), which is 10 times higher than the crude fatality rate of about 0.2% for children with COVID-19 reported in the domestic literature (13). At present, MIS-C is scarcely reported from China, which may benefit from our country's epidemic prevention and control policy of "external input and internal rebound".

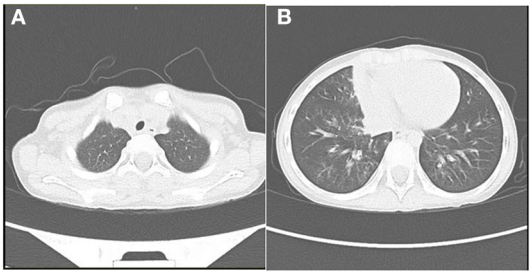

FIGURE 1
Patient's chest CT showed a few inflammatory lesions in the right upper lobe **(A)**, the middle lobe, and the lower lobe of both lungs and the lower lobe of both lungs **(B)** on the first day of admission.

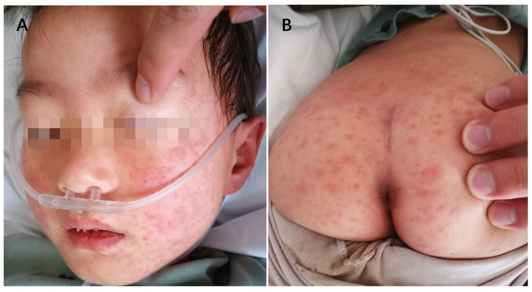

FIGURE 2
Pleomorphic rash of face **(A)** and trunk **(B)**.

Pathogenesis

SARS-CoV-2 infection is typically very mild and often asymptomatic in children. A complication is the rare MIS-C associated with COVID-19, presenting 4–6 weeks after infection as high fever, organ dysfunction, and strongly elevated markers of inflammation (14). Understanding the pathogenesis of MIS-C will be necessary to inform clinical management and prevention efforts. The underlying pathogenesis has not yet been fully elucidated, while an abnormal immune response is blamed as the main factor in the pathogenesis of MIS-C. Virus-induced post-infective immune dysregulation appears to play a predominant role, with MIS-C commonly developing 2–6 weeks after the peak of the COVID-19 epidemic (15). Another studys (4, 5) also reported that the peak onset time of MIS-C is approximately 4–5 weeks behind the regional peak onset of COVID-19, and the delayed onset of symptoms coincides with the time of the acquired immune response, indicating that MIS-C may be a new type of COVID-19 abnormal immune response after infection. The RT-PCR test results for SARS-CoV-2 in some children with MIS-C were positive, and SARS-CoV-2-related IgM and IgG antibodies could also be detected, indicating that MIS-C and SARS-CoV-2 infection are closely related (6, 16). A study also discovered that a cytokine storm occurred in patients with severe COVID-19, accompanied by severe immune function damage (7, 17). Children with MIS-C have markedly increased levels of inflammatory factors such as IL-4, IL-6, IL-12/IL-23, IL-1β, TNF-β, and ferritin (8, 18). In addition, the timing of the interferon (IFN) response to SARS-CoV-2 infection can vary with viral load and genetic differences in host response. When viral load is low, IFN responses are engaged and contribute to viral clearance, resulting in mild infection. When viral load is high and/or genetic factors slow antiviral responses, virus replication can delay the IFN response, and cytokine storm can result before adaptive responses clear the virus, resulting in severe disease including multisystem inflammatory syndrome in children (MIS-C) (9, 19). The pathogenesis of MIS-C may also be related to damage to endothelial damage induced by SARS-CoV-2. Studies have shown that SARS-CoV-2 can invade endothelial cells, leading to endothelial cell damage and thrombosis. Multiple organ damage may be associated with endothelial damage caused by viral infections (10, 20, 21).

Diagnosis

At present, there are 3 preliminary diagnostic criteria for MIS-C in the world. (1) Royal College of Pediatrics and Child Health (UK) preliminary diagnostic criteria (13, 22): pediatric age group, persistent fever, and evidence of single or multiorgan dysfunction (shock, cardiac, respiratory, renal, gastrointestinal, or neurological disorder) with additional features, which may include children fulfilling full or partial criteria for Kawasaki disease. SARS-CoV-2 PCR testing may be either positive or negative. (2) Centers for Disease Control and Prevention (United States) preliminary diagnostic criteria (14, 23): (1) age $<$ 21 years; (2) fever for at least 24 h$\geq$38.0 °C; (3) serious illness leading to hospitalization; (4) 2 or more organ systems affected (e.g., cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, and neurological); (5) positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms. (3) World Health Organization (15, 24): (1) age $<$

19 years old; (2) persistent fever for more than 3 days; (3) at least two concomitant symptoms; (i) rash or bilateral non-purulent conjunctivitis or mucocutaneous signs (oral, hands, or feet); (ii) hypotension or shock; (iii) features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiography findings or elevated troponin/NT-pro-BNP), (iv) evidence of coagulopathy (by PT, APTT, elevated d-dimers), (v) acute gastrointestinal problems (diarrhea, vomiting, or abdominal pain); (4) elevated inflammatory markers, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) or procalcitonin (PCT), serum ferritin, etc; (5) exclusion of inflammation caused by other pathogenic microorganisms; (6) evidence of COVID-19 infection.

Differential diagnosis

Overlapping features between MIS-C and KD make diagnosis challenging. Some patients with MIS-C have features resembling KD, a vasculitis of medium-sized vessels, particularly coronary arteries. Like KD, MIS-C often presents with fever, conjunctivitis, rash, extremity changes, mucocutaneous findings, and adenopathy, but several epidemiological differences suggest distinct pathologic mechanisms. MIS-C is a severe inflammatory syndrome similar to Kawasaki disease (KD), nevertheless, differs from KD in several clinical features. Toxic shock and vasoplegic shock are more common in patients with MIS-C but are unusual in classic KD. Once MIS-C occurs, it often affects multiple systems, most of which are critically ill and can progress and worsen rapidly in a short time. Despite the overlapping features of MIS-C and Kawasaki disease, they appear to have distinct inflammatory pathways.

Age Patients with MIS-C typically affect older children and adolescents, with a median age at presentation of at least 8 years(range = 2 weeks−20 years), whereas KD typically affects infants and young children, with a median age of 2 years (12, 25–28).

Ethnicity Despite high COVID-19 caseloads in Asia, such as China, Korea, and Japan–countries with the highest worldwide KD incidences, MIS-C has been reported rarely in Asia (29). The main affected population is in In several series, MIS-C patients may be seen more often in children of Europe, United States, and African ethnicity (25). It is suggested that the occurrence of MIS-C may be related to ethnicity susceptibility.

Clinical features MIS-C reports, characterized predominantly by shock, cardiac dysfunction, abdominal pain, and markedly elevated inflammatory markers, and almost all had positive SARS-CoV-2 test results, and relatively few classic KD criteria when compared with children with KD. Approximately two-thirds did not have preexisting underlying medical conditions before MIS-C onset (2, 26–28).

Cardiovascular features Although both KD and MIS-C can have cardiovascular involvement, the nature of this involvement appears to differ between the two syndromes. Cardiac features of MIS-C most dramatically show moderate to very severe myocardial involvement (manifested by imaging and strikingly high NT-pro-BNP and troponin levels), much greater than associated with KD or KD shock syndrome, As distinct from KD, left ventricular dysfunction is the predominant cardiac feature in patients with MIS-C and the proportion of cardiac dysfunction in MIS-C was considerably higher. In KD, the cardiac hallmark, of course, is coronary artery abnormalities (2, 26–28).

Laboratory features Laboratory features of MIS-C are also quite distinct from those in KD, with greater resemblance to those of MAS (elevated ferritin, D-dimers, triglycerides) and the cytokine storm of TSS. When comparing laboratory testing, the inflammatory markers are more elevated in MIS-C when compared with KD [most notably c- reactive protein (CRP), ferritin, and D-dimer] and the absolute lymphocyte count and platelets tend to be lower in MIS-C when compared to KD (28).

Inflammatory pathways KD and MIS-C have distinct inflammatory pathways. The inflammatory response in MIS-C differs from the cytokine storm of severe acute COVID-19, shares several features with KD, but also differs from this condition with respect to T cell subsets, interleukin (IL)-17A, and biomarkers associated with arterial damage. Autoantibody profiling suggests multiple autoantibodies that could be involved in the pathogenesis of MIS-C. The inflammation of Kawasaki disease is thought to be mediated by autoantibodies following infection in a genetically predisposed host (2, 14).

Etiology KD etiological studies have confirmed that its pathogenesis is related to viral, bacterial, or mycoplasma infection, while MIS-C is primarily found in areas where SARS-CoV-2 is widespread, along with COVID-19 relevant evidence or relevant epidemiological history.

Treatment

Given that MIS-C has symptoms similar to KD, treatment regimens have been extrapolated from the guidelines for the management of patients with KD. All children meeting the WHO case definition criteria for MIS-C should be monitored in the hospital with possible admission to the PICU and early involvement of a multidisciplinary team. Rapid and aggressive treatment options should be considered according to the evolution of the disease.

Currently, the treatment of MIS-C refers to the American College of Rheumatology clinical guidelines for SARS-CoV-2-associated multisystem inflammatory syndrome in children (30–32). Current practices and published guidelines for the treatment of MIS-C support the use of intravenous immunoglobulin (IVIG) and/or corticosteroids as a first-line cornerstone of therapy (33). In addition to antithrombotic therapy and second-line treatment with different immunomodulatory drugs (e.g., tumor necrosis factor inhibitor, interleukin-1 inhibitor, or

interleukin-6 inhibitor), other supportive therapeutic agents are concomitantly used (34, 35). Consensus guidelines support the use of high-dose IVIG for MIS-C patients, administered in a single dose at 2 g/kg based on ideal body weight (max 100 g), which can sometimes be used as needed before full diagnostic evaluation is completed. Glucocorticoids are used in low doses as adjunctive therapy in patients with the moderate-to-severe disease or high doses as intensification therapy in patients with the refractory disease (7, 36). Currently, the treatments of MIS-C are based on treatment strategies in KD or COVID-19, and it is reported some new treatment strategies are effective, such as treatments related to pathogenesis. Anti-cytokine therapy such as anakinra, tocilizumab, or infliximab has been used in cases refractory to first-line treatments (37). An accurate assessment is the first step of antithrombotic therapy, Aspirin is used as a thromboprophylaxis in MIS-C, low-dose aspirin (3–5 mg/kg/day, maximum 81 mg/day). In addition to aspirin, the concomitant use of anticoagulation, such as low molecular weight heparin.

Conclusions

In conclusion, MIS-C is a hyper-inflammatory syndrome affecting multiple organs and is triggered by SARS-CoV-2 infection, high titres of anti-SARS-CoV-2 antibodies are seen in these patients. MIS-C develops following SARS-CoV-2 infection, and presumably, those adaptive immune mechanisms have a major role to play in the pathogenesis of this condition. Although clinical manifestations of MIS-C and KD may be overlapping, some of the clinical manifestations of MIS-C mimic KD shock syndrome, these appear to be two distinct clinical entities, it is the cardiovascular manifestations that are most prominent. MIS-C cases have now been reported from several countries the world over, while it has been rarely reported in China, which should be closely related to China's scientific epidemic prevention and control measures, Other possible causes need further study. Current treatment guidelines recommend the use of intravenous immunoglobulin (IVIG) and/or corticosteroids as the first-line cornerstone of therapy for MIS-C, However, the new therapy will be used as in cases refractory to first-line treatments need to be further studied. COVID-19 is still a pandemic around the world, and the pathogenesis, diagnostic standard, and treatment of MIS-C require further research and exploration. More attention should be paid to children with COVID-19-related evidence or epidemiological history.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

Guarantor of integrity of the entire study: W-yW and Y-jW. Study concepts: Y-jW. Study design: C-xA. Definition of intellectual content: HL. Literature research: BY. Data acquisition: W-yW. Data analysis: Q-jZ. Statistical analysis: S-yW and W-yL. Manuscript preparation: W-yW. Manuscript editing: W-yW, Y-jW, and HL. Manuscript review: Y-jW and HL. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

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Increased expression of the TLR7/9 signaling pathways in chronic active EBV infection

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We aimed to investigate the immunological mechanisms of the Toll-like receptor (TLR) signaling pathways in different types of Epstein-Barr virus (EBV) infection. We retrospectively summarized the clinical data, routine laboratory tests and the immunological function of the infectious mononucleosis (IM) and chronic active EBV infection (CAEBV) patients. A real-time quantitative PCR array was used to detect the mRNA expression levels of TLR7/TLR9 and myeloid-differentiation factor 88 (MyD88). Flow cytometry was used to detect the protein expression of TLR7/TLR9. The MyD88 and nuclear factor-κB (NF-κB) (p65) protein were detected by western blotting. A cytometric bead array (CBA) assay was used to detect the expression of downstream cytokines. CAEBV patients presented with increased expression of TLR7/TLR9 in monocytes and B lymphocytes. TLR9 expression in the B lymphocytes of IM patients was decreased compared with the CAEBV pateints. Downstream signaling mediators, including MyD88 and NF-κB, were revealed to be increased in EBV-infected patients. Moreover, the expression of MyD88 and NF-κB was higher in CAEBV patients, leading to disrupted balance of downstream cytokines. EBV may activate the immune system *via* TLR7/TLR9 signaling pathways. Moreover, the overactivated TLR7/TLR9 pathway in CAEBV patients resulted in excessive inflammation, which might be relevant to the poor prognosis.

KEYWORDS

epstein-Barr virus, chronic active EBV infection, immune response, toll-like receptor, excessive inflammatory response

Introduction

Epstein-Barr virus (EBV), a human gammaherpesvirus, infects 90%–95% of the world's human population. EBV infection has been found to be involved in the development of various human diseases from asymptomatic to malignant forms. Infectious mononucleosis (IM), one of the benign diseases associated with EBV infection, mainly causes transient fever, lymphadenopathy, and pharyngitis manifestations (1). Rare EBV-infected individuals without apparent immunodeficiency present with chronic active EBV infection (CAEBV), which is characterized by persistent or recurring IM-like symptoms, lymphadenopathy and in some patients hypersensitivity to mosquito bites and hydroa vacciniforme-like eruption of high EBV-DNA load in peripheral blood mononuclear cells (2). This disease can present with persistent or even fulminant expression with death and a poor prognosis (1).

Most notably, EBV-associated diseases are relatively different in infected individuals, which might result from the presence of effective immune responses against EBV (3, 4).

Toll-like receptors (TLRs) can recognize EBV and initiate immune responses, which lead to the production of inflammatory cytokines and antiviral mediators. TLR7 and TLR9 are mainly expressed in intracellular vesicles. TLR7 is able to recognize EBV single-stranded RNA (ssRNA), whereas TLR9 recognizes EBV unmethylated 2′-deoxyribo (cytidine-phosphate-guanosine) (5). Myeloid differentiation factor 88 (MyD88) is recruited by TLRs and subsequently activates the transcription factor nuclear factor-κB (NF-κB) (6). NF-κB, as a transcription factor of REL family members, mediates inflammatory and antiapoptotic molecular signals. After activation, NF-κB can translocate to the nucleus and bind DNA to regulate downstream gene expression (2, 7, 8). Interactions between EBV and TLR signaling pathways might affect EBV infection and host antiviral immunity (5).

The molecular mechanism of TLR signaling pathways against EBV infection also remains to be fully revealed. In this study, we investigated the role of TLR signaling pathways in different EBV-associated diseases. Our results demonstrate that TLR7/9-MyD88-NF-κB pathways are overactivated in CAEBV patients, leading to excessive cytokine production.

Methods

The study was approved by the Ethics Committee of the Children's Hospital of Fudan University. The patients and their parents provided written informed consent for enrollment in this study. The clinical trial registration number is NCT03374566 (12/12/2017-03/11/2021).

Patients and clinical data

We included 10 healthy control children without EBV infection (ang-matched healthy control group), 20 children with acute infectious mononucleosis (IM group), and 10 children with CAEBV without a clear genetic defect (CAEBV group) in the study. The gender (M/F) of the groups was balanced. We summarized the clinical data and some related laboratory examinations of the patients.

TLR7/9 signaling pathway detection by quantitative real-time PCR and flow cytometry

For real-time PCR, PBMCs were prepared from EDTA-treated whole blood from 10 healthy controls, 20 IM patients, and 10 CAEBV patients as previously reported (9). Total RNA was extracted from PBMCs using RNAiso Plus Reagent (TaKaRa, Japan), and cDNA was acquired using a reverse transcription kit (Qiagen, USA) following the manufacturer's instructions. Real-time RT-PCR was performed using Takara SYBR Fast qPCR Mix (Takara, Japan) on a LightCycler 480 Instrument II (Roche, Switzerland). The primer sequences were as follows: TLR7 (Forward): TGCTGTGTGGTTTGTCTGGT, TLR7 (Reverse): GCCCCACACAAGTCACATCT; TLR9 (Forward): CTGGCTG TTCCTGAAGTCTGTGC, TLR9 (Reverse): GTGGATGCGGTT GGAGGACAAG; MyD88 (Forward): AGTGGGATGGGGAG AACAGA, MyD88 (Reverse): TGTAGTCCAGCAACAGCCAG. Fold changes in the patients vs. healthy controls were analyzed using the $2^{-\Delta\Delta CT}$ method. The β-actin gene was used as an inner reference.

For PhosFlow staining, we used a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) for analysis (9). Whole blood was used for staining for lymphocyte surface markers after red cell lysis and analysis according to a standard multicolor flow cytometric protocol with appropriate fluorochrome-labeled monoclonal antibodies or isotype-matched control antibodies. After being washed twice with PBS, 1×104 to 5×104 live cells were analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA) using FACSDiva software (BD Biosciences). Whole blood was permeabilized with Perm Buffer III according to the manufacturer's instructions (BD Biosciences). An APC-H7-conjugated antibody against CD3, a BV510-conjugated antibody against CD4, a PercP-Cy5-5-conjugated antibody against CD8, a BV421-conjugated antibody against CD14, and a PercP-Cy7-conjugated antibody against CD19 were used to gate lymphocyte subsets. For the Flow analysis, we used the following antibodies: PE-conjugated antibody against TLR7 and APC-conjugated antibody against TLR9 (all from BD Biosciences).

Western blotting

As previously reported (10), cytoplasmic and nuclear proteins were extracted using NE-PERNuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, USA) following the manufacturer's instructions. Equal amounts of cytoplasmic or nuclear extracts were separated on 12% SDS polyacrylamide gels and transferred to PVDF membranes. Blots were probed with primary antibodies against MyD88, NF-κB p65, β-actin, or histone H3 (Cell Signaling Technology, Beverly, MA). Primary antibodies were detected with horseradish peroxidase-conjugated secondary antibody. Visualization was performed using an ECL peroxidase substrate.

Cytokine detection

We used a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) to measure cytokine expression using

a BD Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kit (11). We first added 50 µl of flex set standard dilutions to the first 8 tubes (no standard dilution, 1:729, 1:243, 1:81, 1:27, 1:9, 1:3 and top standard). We then added 50 µl of each unknown sample to the appropriate assay tubes and added 20 µl of the mixed capture beads to each assay tube. The tubes were incubated for 2 h at room temperature. Twenty microliters of the mixed Human Detection Reagent (Part A) were added to each assay tube. The tubes were incubated for 2 h at room temperature. We added 1 ml of wash buffer to each assay tube with centrifugation at 200 g for 5 min. Then, we added 100 µl of enhanced sensitivity detection reagent (Part B) to each assay tube with gentle mixing of the tubes. The tubes were incubated for 1 h at room temperature. Then, 300 µl of wash buffer was added to each assay tube with brief vertexing to resuspend the beads. All reagents were from BD Biosciences. Finally, we used FCAP array software to analyze the data.

Statistical analysis

The data were analyzed using Student's *t*-tests, Welch tests, or nonparametric Mann–Whitney *U* tests with GraphPad Prism software; *p*-values <0.05 were considered significant.

Results

Clinical characteristics

The clinical manifestations of children with EBV infection are shown in **Supplementary Table S1**. Fever is the most common clinical manifestation. Thirty children (100%) with EBV infection all presented with fever. Lymphadenopathy (95% IM vs. 80% CAEBV), hepatomegaly (80% IM vs. 60% CAEBV), and splenomegaly (80% IM vs. 60% CAEBV) were observed in the patients. Among the patients, ten IM patients (50%) with hepatomegaly presented with liver dysfunction, while the six CAEBV patients described above all (100%) had liver dysfunction. Bilateral eyelid edema was observed in 11 (55%) IM children, whereas none was observed in the CAEBV group. Two CAEBV children (P25 and P30) developed hemophagocytic syndrome presenting with continual fever and pancytopenia.

Laboratory examinations

EBV VCA IgM in blood serum was positive in all IM patients, whereas only one CAEBV case was positive for EBV VCA IgM. On the other hand, seventeen children in the IM group (85%) had elevated EBV VCA IgG, and in the CAEBV group, there were nine patients (90%). Moreover, the increase in the EBV VCA IgG level in the CAEBV children (mean = 153.44 ± 65.2) was much greater than in the IM children (mean = 83.7 ± 52.4). We found that in thirteen IM patients (65%), the number of white blood cells (WBCs) was increased, whereas the levels of WBCs in six CAEBV children (60%) were decreased.

The immunological characteristics of the patients are listed in **Supplementary Table S2**. We observed that EBV-infected patients presented with elevated IgG and IgE levels (70% IM vs. 70% CAEBV, 30% IM vs. 30% CAEBV, respectively). However, the CAEBV patients had significantly higher IgE levels, of more than 1,000 KU/L. Increased CD8+ T lymphocytes (95%) and decreased CD4+ T lymphocytes (70%)/B lymphocytes (90%)/NK cells (80%) were observed in the IM patients. Compared with the children in the IM group, the counts of T lymphocytes were significantly lower in the CAEBV group. The proportion of CD8+ T cells in four CAEBV children (40%) was increased, while it was in the normal range in 5 patients (50%). Importantly, nine patients (90%) had a reduced proportion of B lymphocytes. With regard to NK cells, a reduced proportion was observed in four patients (40%), while an increased proportion was observed in three patients (30%).

Expression of the TLR7/TLR9 - MyD88-Nf-κB/IRF7 signaling pathway

TLR7 and TLR9 expression levels were increased in CAEBV infection

Real-time PCR and flow cytometry analyses were performed to determine whether the expression of TLR7 and TLR9 was changed. We found that TLR7 and TLR9 transcriptional levels were significantly increased in peripheral blood mononuclear cells (PBMCs) of CAEBV patients compared with IM patients and healthy controls (**Figures 1A**, **2A**). We therefore checked whether TLR7 and TLR9 protein levels were also altered in EBV-infected individuals. Moreover, the TLR7 and TLR9 protein levels were in accordance with the transcriptional levels (**Figures 1B**, **2B**). The results suggested that TLR7 and TLR9 were activated in CAEBV patients. However, TLR7 and TLR9 expression in IM patients was downregulated in PBMCs. Flow cytometry analysis showed that the TLR7 and TLR9 protein levels were in accordance with the mRNA changes (**Supplementary Figure S1A,D**).

Moreover, we separately stained the surface markers CD19 and CD14 to gate B lymphocytes and monocytes and then investigated the expression of TLR7 and TLR9. We found that CAEBV patients presented with increased expression of TLR7 in CD14+ monocytes and CD19+ B lymphocytes (**Figure 2C, D**), whereas its expression in IM patients decreased compared to the CAEBV patients (**Figure 1C**). TLR9 expression in B lymphocytes and monocytes was significantly higher in CAEBV patients than in IM patients (**Figure 1D**). Our data demonstrated that the expression of TLR9 was downregulated in B lymphocytes of children with IM, whereas it was

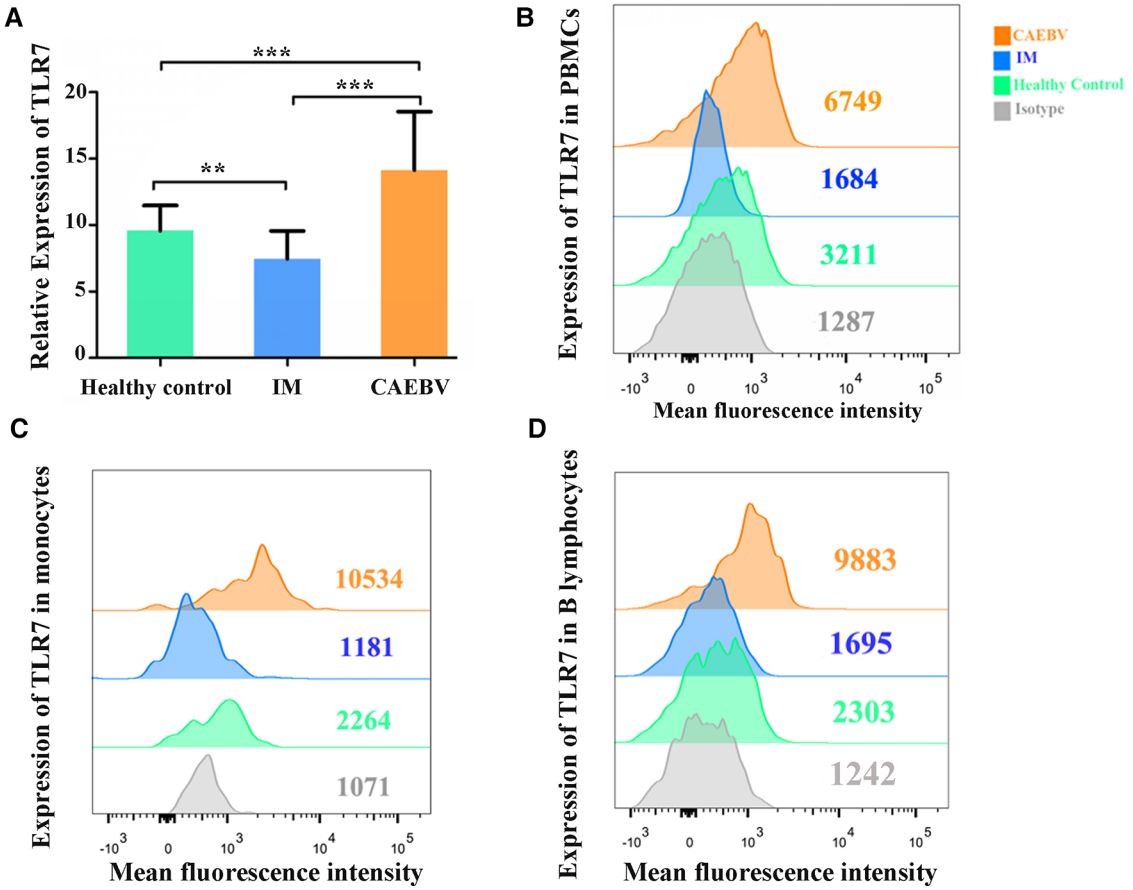

FIGURE 1
TLR7 expression is associated with EBV infection. (A) MRNA accumulation of TLR7 was measured in PBMCs from healthy controls (age matched, $n = 20$), IM patients ($n = 20$) and CAEBV patients ($n = 10$). (B) Flow cytometry analysis showed the levels of TLR7 protein. (C) The expression of TLR7 in CD19+ B lymphocytes by flow cytometry analysis. (D) The expression of TLR7 in CD14+ monocytes by flow cytometry analysis. (**$p < 0.01$ and ***$p < 0.001$ by Duncan's test).

overexpressed in CD14+ monocytes (**Supplementary Figure S1**). These observations suggest that EBV influenced TLR9 expression, possibly depending on the cell type.

Activation of the TLR7/9 downstream signaling pathway

Signaling of TLR7 and TLR9 proceeds through MyD88, leading to the activation of the NF-κB signaling pathway (6). Western blot analysis showed that the expression levels of MyD88 were increased in EBV-infected patients compared with healthy controls. Furthermore, MyD88 expression in CAEBV patients was higher than that in IM patients, suggesting stronger activation of MyD88 in CAEBV than in IM patients (**Figure 3A,B**).

Once activated, the free NF-κB molecular subunits, for example, p50 and p65, translocate into the nucleus to regulate the expression of multiple target genes involved in cell activation and the production of inflammatory cytokines (12, 13). To demonstrate the activation of the NF-κB signaling pathway, we monitored the expression of NF-κB p65 in the cell nucleus. As shown in **Figure 4A**, we detected that the p65 level in nuclei was significantly higher in EBV patients than in healthy controls. Furthermore, the CAEBV patients presented with prominently upregulated NF-κB p65 compared with the IM patients. These results indicated that the NF-κB signaling pathway was activated in EBV-infected patients, particularly in CAEBV patients.

Increased expression of cytokines after EBV infection

Once TLRs are activated, immunocytes rapidly produce a broad range of inflammatory cytokine responses against EBV infection (5, 14). Consistent with the aforementioned laboratory results, we detected cytokines such as IL-6, IL-8, IL-10 and IFN-γ in the serum. The expression levels of cytokines in EBV-infected patients were observed to increase compared with those in healthy controls (**Figure 4C**). In addition, the expression

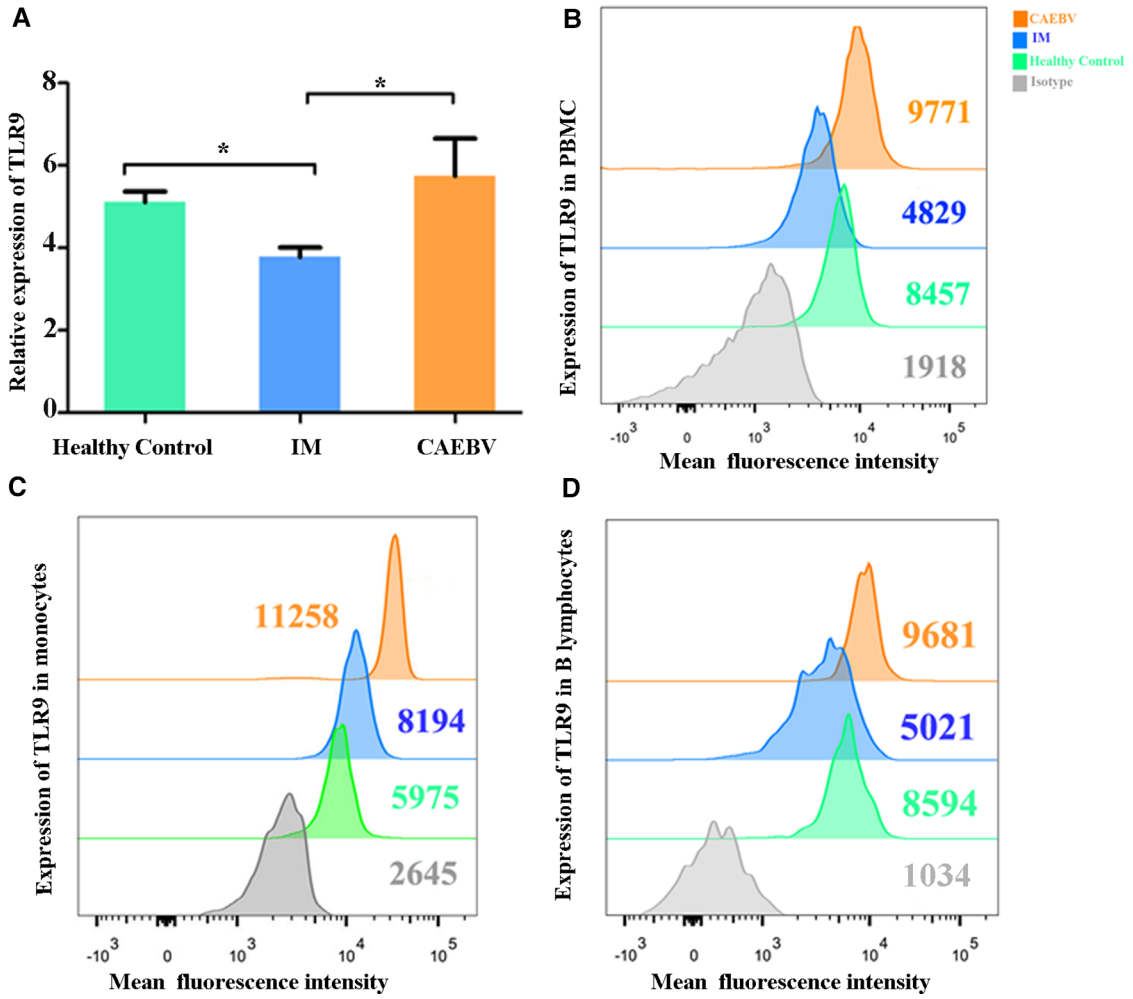

FIGURE 2
TLR9 expression is associated with EBV infection. (**A**) The level of TLR9 mRNA was measured in PBMCs from healthy controls (age matched, $n = 20$), IM patients ($n = 20$) and CAEBV patients ($n = 10$). (**B**) Flow cytometry analysis showed the levels of TLR9 protein. (**C**) The expression of TLR9 in CD19+ B lymphocytes by flow cytometry analysis. (**D**) The expression of TLR9 in CD14+ monocytes by flow cytometry analysis. (*$p < 0.05$ by Duncan's test).

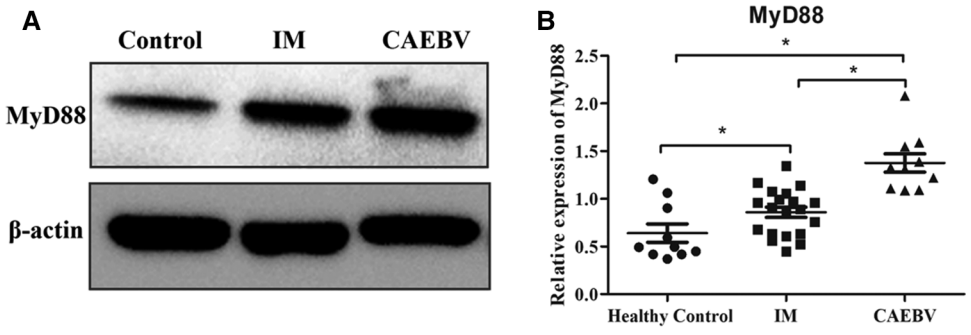

FIGURE 3
Myd88 expression in the EBV-infected patients. (**A**) The protein level of MyD88 in the cytosolic fractions derived from the healthy control, IM patient and CAEBV patient. (**B**) The protein level of MyD88 in healthy controls (age matched, $n = 20$), IM patients ($n = 20$) and CAEBV patients ($n = 10$). (*$p < 0.05$ by Duncan's test).

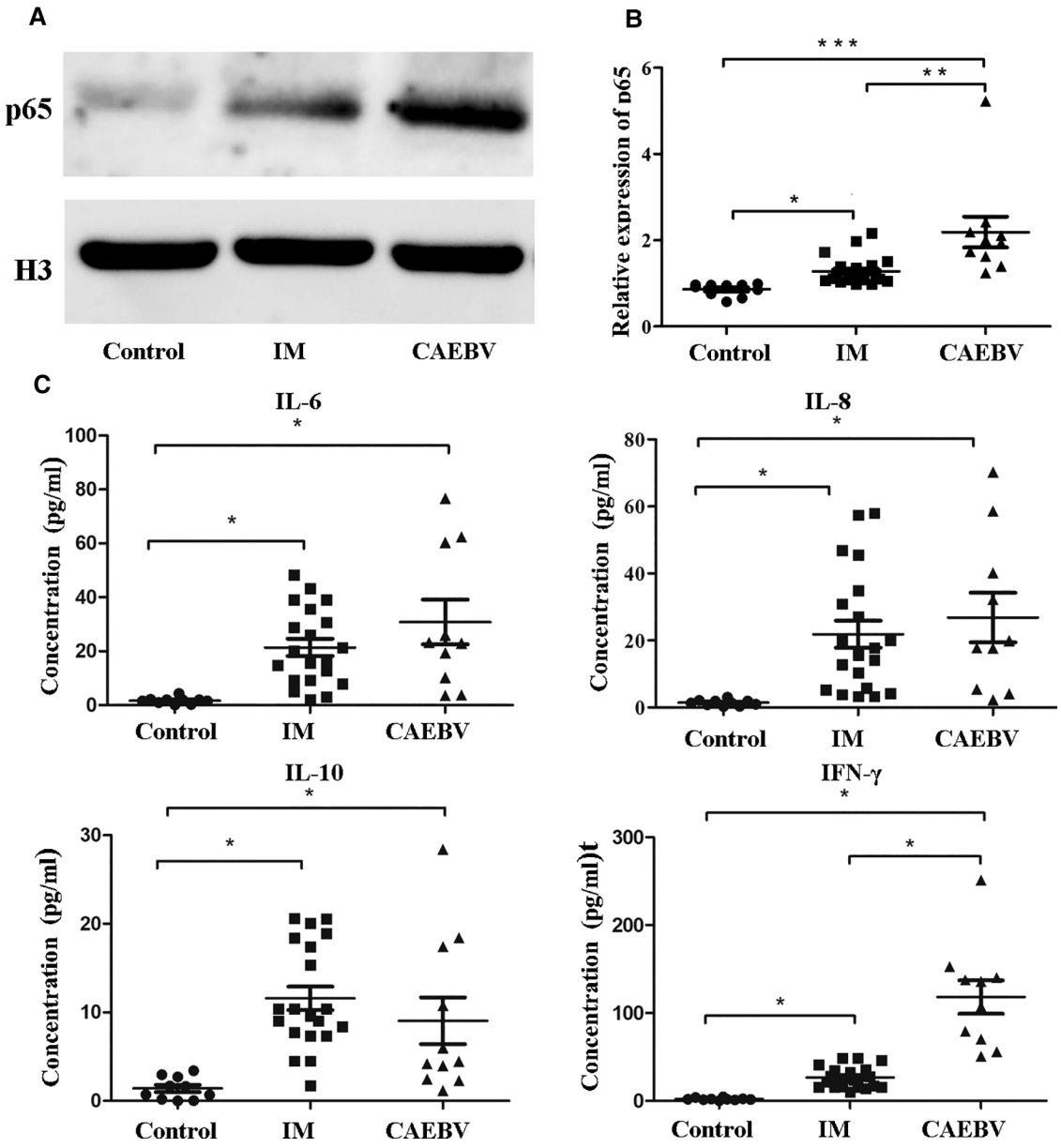

FIGURE 4
NF-κB signaling activation in EBV-infected patients. (**A**) The protein levels of p65 in the nucleus by Western blot analysis. (**B**) The protein level of p65 in the nucleus in healthy controls (age-matched $N = 10$), IM patients ($n = 20$) and CAEBV patients ($n = 10$). (**C**) Cytometric bead array (CBA) by flow cytometry showed that the expression levels of downstream cytokines, such as IL-6/IL-8/IL-10 and IFN-γ, were measured in serum obtained from healthy controls (age matched, $n = 20$) and IM patients ($n = 20$) and CAEBV patients ($n = 10$). (*$p < 0.05$ **$p < 0.01$ and ***$p < 0.001$ by Duncan's test).

levels IFN-γ in the CAEBV group were observed to be significantly greater than those in the IM group.

Discussion

Epstein-Barr virus (EBV), a human herpesvirus, maintains a finely balanced relationship with the host; thereafter, most carriers are asymptomatic during their lifetime. Some individuals occasionally develop IM, one of the benign and acute diseases, after infection with EBV. Moreover, a minority of EBV-infected individuals present with CAEBV characterized by a poor prognosis (1). Large expansions of CD8+ T lymphocytes are detected to control EBV infection during IM (15). We observed that CD8+ T lymphocytes were significantly expanded in children with IM. In addition, in CAEBV patients, EBV-specific CD8+ T lymphocytes and NK cells were detected to be lower than those in IM patients, making it difficult to control EBV infection. CD8+ T lymphocyte

and NK cell deficiency might be associated with loss of control of EBV infection. The CAEBV children showed higher expression of EBV-VCA IgG than the IM children, which might result from continuously uncontrolled EBV infection.

The interaction between EBV infection and the immune system by TLRs plays a critical role in the outcome of the infection. The present study (5) demonstrated that EBV can develop complex strategies to interact with TLR signaling pathways. NF-κB, the transcription factor related to innate immune response genes, has recently been found to be overexpressed in cells infected with EBV (2, 16). Appropriate production of cytokines might be beneficial for EBV infection; however, excessive cytokines might have a negative impact on EBV-infected hosts. Dysregulation of TLR-mediated immune responses might be related to autoimmunity or autoinflammatory diseases by inducing abnormal activation of host immunity (17). The expression of TLR7 and TLR9 signaling pathways can be elicited or modulated by EBV infection (17). The role of the TLR signaling pathway in the fate of EBV infection has remained undermined. Farina et al. (18) found that expression of the EBV lytic gene BFRF1 was associated with a trend of TLR7 downregulation. In vitro studies showed that EBV can upregulate the expression of TLR7 and MyD88 in B lymphocytes (19). In our study, we evaluated the role of TLR7 and TLR9 in different EBV infections. Our results demonstrated that TLR7 expression levels in CAEBV patients were significantly increased, including CD14+ monocytes and CD19+ B lymphocytes, whereas TLR7 expression in IM patients was decreased. Therefore, we presume that the different stages of EBV-related proteins might affect the expression level of TLR7. In contrast, TLR7 expression in CAEBV patients remained high, which might be relevant to individual CAEBV specificity. The same phenomenon occurs in the expression of TLR9. Compared with children in the IM group, the expression of TLR9 in children with CAEBV was increased, both in monocytes and B lymphocytes. However, our data demonstrated that the expression of TLR9 was downregulated in B lymphocytes in children with IM, whereas it was overexpressed in CD14+ monocytes. These results indicated that EBV infection leads to an increase in TLR9 expression in monocytes. The impact of EBV infection with different TLR expression might depend on cell and individual specificity. Van Gent et al. identified that the EBV lytic-phase protein BGLF5 can downregulate TLR9 levels *via* RNA degradation *in vitro* (20). In addition, Fathallah et al. found that EBV latent membrane protein 1 (LMP1) can prevent TLR9 promoter deregulation and inhibit TLR9 transcription by activating the NF-κB pathway in B lymphocytes (21). Our data demonstrated that MyD88 and NF-κB are overexpressed in EBV-infected individuals compared to controls. TLR7 and TLR9 signaling themselves mediate NF-κB activation, inducing a negative feedback loop for TLR9 expression in B lymphocytes. Thus, EBV-induced NF-κB activity might be the source of TLR9 downregulation in B lymphocytes, which might be related to cell specificity. Moreover, TLR9 can physically interact with TLR7 and inhibit TLR7 function in a dose-dependent manner (22). However, CAEBV patients developed persistently excessive activation of TLR7 and TLR9 and its downstream signaling mediators. Our present results indicate that CAEBV patients might develop deficiency in the self-regulation of TLR negative feedback.

NF-kB is constitutively activated in many types of viral and nonviral-associated human cancers. Activation of the NF-κB signaling pathway induces NF-kB nuclear translocation and affects the expression of genes encoding key players in cell survival, cellular proliferation, and the immune response, which result in the production of downstream cytokines. As a result, its downstream signaling cytokines were upregulated. The upstream molecules TLR7 and TLR9, whose expression is upregulated in CAEBV patients, might play indirect roles in NF-κB activation. Previous studies have found that EBV upregulates the expression of IL-6, IL-8 and IFN-γ by activating the TLR9 signaling pathway (4, 23). IL-10, as an immunosuppressive factor, is mainly secreted by monocytes and Th1-type cells. It can regulate cell growth and differentiation and participate in a variety of inflammatory and immune responses. TLR9 has been found to promote the differentiation of T cells into Th1 cells and participate in the synthesis and secretion of IL-10. Moreover, TLR9 is also involved in the process of secretion of IFN-γ by T lymphocytes (24). Excessive activation of NF-κB signaling has been linked to human inflammatory diseases (25). We observed that high levels of human cytokines, including IL-6, IL-8, IL-10, and IFN-γ, were detected in the blood serum of EBV-infected patients. Moreover, the expression of IL-10 and IFN-γ increased significantly in CAEBV patients. The TLR signaling pathway, which is related to the secretion of multiple cytokines, plays complicated roles in EBV infection. The changes in the adaptor MyD88 and mediator NF-κB were inconsistent with TLR expression in IM patients. We suspected that, on the one hand, in the early stage of EBV infection, TLR expression was upregulated, which then downregulated negative feedback. On the other hand, EBV leads to increased expression of MyD88 and NF-κB *via* other signaling pathways. Our results demonstrated that CAEBV patients maintain excessive inflammatory conditions, mirroring hypercytokinemia characteristics of CAEBV patients. TLR7 and TLR9 signaling pathways may have distinct outcomes depending on the cell types expressing them. They are critically involved not only in the activation of immunocytes but also in cytokine induction.

Both chronic infection and excessive inflammation contribute to the occurrence and development of tumors (26). Nakamura et al. reported that the expression of cytosine deaminase (AID), which participates in somatic hypermutation and the transformation of immunoglobulin classes, was upregulated in CAEBV patients. The dysregulation of AID

leads to gene mutations and B-cell lymphoma (2). NF-κB signaling has been found to induce the expression of AID (27). Therefore, overactivation of the TLR-NF-κB pathway may play a certain role in the development of EBV-related lymphoma. Dysregulated AID expression induces genomic mutation, leading to the development of B cell lymphoma. Interestingly, NF-κB can induce AID expression in B cells. The activation of NF-κB in CAEBV patients may lead to the upregulation of AID, which might be prone to develop lymphomagenesis.

Conclusion

Our study provides new insights into the possible involvement of TLR7 and TLR9 in the pathogenesis of CAEBV, and TLR7/9-dependent immune responses to EBV might contribute to the long-term inflammatory response in CAEBV patients. Here, we provide evidence that excessive inflammatory responses meditated by overexpression of the TLR7/TLR9-MyD88-NF-κB signaling pathway might contribute to the poor prognosis of CAEBV. Nevertheless, additional mechanistic investigations are need to definite how the TLR7/TLR9-MyD88-NF-κB signaling pathway becomes over activated.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by The study was approved by the Ethics Committee of the Children's Hospital of Fudan University. The patients and their parents provided written informed consent for enrollment in this study. The clinical trial registration number is NCT03374566. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

LL designed and performed the research. LL, YW and BS analyzed data. WW and WJ provided clinical data and advice. LL wrote the paper. XW provided advice on the imaging and paper. JS finally approve the version to be submitted. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1091571/full#supplementary-material.

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A global bibliometric analysis on Kawasaki disease research over the last 5 years (2017–2021)

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Background: Kawasaki disease (KD) is a systemic vasculitis of unknown etiology that mainly affects children. We aim to conduct a bibliometric analysis to explore the latest research hotspots and trends of KD.

Method: By using the keywords "Kawasaki disease," "Kawasaki syndrome", and "Mucocutaneous Lymph Node Syndrome," the Web of Science (WOS) and Scopus databases were searched for publications related to KD from 2017 to 2021. Author, country and journal submissions were classified and evaluated using Biblioshiny software (using R language). VOSviewer (version 1.6.18) was used to visualize the relevant network relationships.

Result: According to the search strategy, 5,848 and 6,804 KD studies were published in WOS and Scopus, respectively. The results showed an overall increasing trend in the number of publications and citations during the study period. The three most influential institutions in the WOS were St. Marianna University, Kawasaki Medical School and The University of Tokyo in Japan, while in Scopus, Harvard Medical School, University of California and Tehran University of Medical Sciences were the most influential institutions. The most influential authors of the two databases are Goodman SG, Kazunori Kataoka, and Takeshi Kimura of the WOS and Marx Nikolau and Wang Y, Burns JC, and Newburger JW of the Scopus, respectively. And Scientific Reports and Frontiers in Pediatrics were the most critical journals. The most cited documents were the WOS document by McCrindle et al. and the Scopus document by Benjamin et al. published in 2017, while the keywords in the last few years were focused on "COVID-19," "multisystem inflammatory syndrome," and "pandemic."

Conclusion: This bibliometric analysis summarizes for the first time the research progress in KD (2017–2021), providing a qualitative and quantitative assessment of KD research bibliometric information. In the field, researchers mainly from Japan and USA are dominant, followed by China. It is recommended to pay close attention to the latest hot spots, such as "COVID-19" and "multisystem inflammatory syndrome." These results provide a more intuitive and convenient way for researchers to obtain the latest information on KD.

KEYWORDS

Kawasaki disease, COVID-19, Web of Science, Scopus, bibliometric analysis

Background

Kawasaki Disease (KD) was first discovered in Japan in 1967 and subsequently reported in dozens of countries around the world. KD is mainly characterized by systemic inflammation, affecting children under 5 years of age, and the incidence of KD has racial and seasonal differences (1). KD can be triggered by infection of various pathogenic microorganisms (2). Genetics may also play an important role in the pathogenesis of KD (3). Studies have found that genetic abnormalities in several different functional types of genes may increase the risk of KD, for example, enhancing T cell activation (ITPKC, ORAI1, and STIM1), dysregulation of B cell signaling (CD40, BLK, and FCGR2A), inhibiting apoptosis (CASP3), and changing transforming growth factor-β signaling (TGFB2, TGFBR2, MMP, and SMAD), etc. (4). The clinical diagnosis of classical KD requires the presence of at least four of the following five clinical features when fever of at least 5 days is satisfied: (i) diffuse congestion and chancre in the lips and mouth; (ii) bilateral conjunctiva in a congested state without exudates; (iii) rash including maculopapular rash, erythema multiforme, etc.; (iv) erythema of the hands and feet in the acute phase and/or subacute peri-finger and toe molting; and (v) enlarged lymph nodes (at least 1.5 cm in diameter), mostly unilateral (5).

With the epidemic of COVID-19, a Kawasaki-like disease has gradually attracted the attention of clinicians and scientists (6, 7). Guidance from the Royal College of Pediatrics and Child Health states that children with (i) persistent fever >48 h, lymphomegaly and evidence of single or multi-organ dysfunction; and (ii) exclusion of any other microorganism causing the disease can be considered for the diagnosis of KD-like multi-inflammatory syndrome (8). Some of the SARS-CoV-2 infected children diagnosed with multi-system inflammatory syndrome (MIS) exhibit clinical features that meet the criteria for Kawasaki disease (9). The first major UK case series study on children with MIS found that about 10–20% of children were eligible for Kawasaki disease diagnosis (10). In contrast, the results of an Italian national multicenter survey showed that children with Kawasaki-like disease had a higher rate of positive SARS-CoV-2 detection (75,5%) compared to children with KD (11).

Research gaps based on literature review

Integrating and analyzing the scientific results of a field of study has always been a priority for scientists, and this has given rise to systematic reviews and meta-analyses, a method of data analysis that integrates different results of similar studies. Using the year of publication as a cut-off point provides a more convenient and macroscopic view of the focus of Kawasaki disease research in each year. A search of the PubMed database revealed several systematic reviews and meta-analyses of research on KD published between 2017 and 2021.

A systematic review assessing the application of steroids for coronary complications in KD was published online by the Cochrane Collaboration in 2017 (12). The study included a total of seven high-quality randomized controlled studies and noted that steroid application in the acute phase of KD improved coronary lesions and significantly reduced inflammatory marker levels. And people in Asia, with high-risk scores and prolonged steroid application, are more likely to be benefiting from steroid use. The study was resubmitted in an updated version in 2022. This updated systematic review included 1 new randomized controlled study in addition to the original study and concluded that the use of corticosteroids also reduced the duration of clinical symptoms (13).

Li et al. (14) published a meta-analysis of predictors of intravenous immunoglobulin-resistant (IVIG-resistant) KD in children in 2018, which included 28 studies totaling 4,442 children with IVIG-resistant KD. This meta-analysis summarized the clinical characteristics of all children as well as certain laboratory test indices. Ultimately, the initial administration of IVIG within 4.0 days after the onset of symptoms, increased clots in blood sedimentation, decreased hemoglobin and platelet counts, oral mucosal lesions, enlarged cervical lymph nodes, swollen extremities and polymorphic rash were found to be risk factors for IVIG resistance. In contrast to previous meta-analyses predicting KD risk factors, this study newly added clinical characteristics, duration of initial drug administration, and hemoglobin concentration as risk factors involved in resistant KD. In the same year, Xie et al. (15) identified new potential genetic biomarkers of KD by meta-analysis, in which 62 genes including genetic polymorphisms of ACE, BLK, CASP3, CD40, FCGR2A, FGβ, HLA-E, IL1A, IL6, ITPKC, LTA, MPO, PD1, SMAD3, CCL17, and TNF might be associated with KD susceptibility, and genetic variants in 47 genes including BTNL2, CASP3, FCGR2A, FGF23, FGβ, GRIN3A, HLA-E, IL10, ITPKC, and TGFBR2 may be associated with the incidence of coronary artery lesions in KD.

In 2019, Tanoshima et al. (16) included 20 clinical studies and attempted a systematic review and meta-analysis in order to assess the effectiveness of antiplatelet agents in the treatment of KD. The antiplatelet agents included in the studies were mainly aspirin, flurbiprofen, dipyridamole, and choline salicylate. However, quantitative synthetic analysis was ultimately not performed due to the high heterogeneity of the included studies and the lack of quantitative data. This study concluded that although the application of antiplatelet agents can inhibit platelet aggregation, strong evidence for the effectiveness of antiplatelet therapy is lacking. In addition, to clarify the efficacy and safety of TNF-α blockers (i.e., infliximab and etanercept) for the treatment of children with KD, a new systematic review article was published online by the Cochrane Library in 2019 (17). The study conclusively determined that

lower quality evidence suggests that the application of TNF-α blockers is beneficial in drug-resistant KD. However, due to the lack of larger, high-quality studies, the above conclusions need to be treated with caution. Meanwhile, another systematic review was conducted by Crayne et al. (18) on the efficacy and safety of second-line therapy (including a second intravenous IVIG, methylprednisolone and infliximab) in patients with refractory Kawasaki disease in patients with drug-resistant KD. This study concluded that infliximab monotherapy should be considered more as second-line therapy in children who do not respond to first IVIG treatment and that it is more effective than second IVIG in relieving febrile symptoms in children.

Two systematic reviews exploring the dose of aspirin therapy in KD were published online in 2020, respectively (19, 20). Interestingly both studies suggest that the application of low doses of aspirin in the acute phase of KD may benefit the child in the treatment of KD. Jia et al. (19) concluded that the application of low-dose aspirin is comparable to high-dose aspirin therapy with less severe side effects, while Chiang et al. (20) stated that prescribing low or no aspirin in the acute phase of KD was strongly associated with a low incidence of coronary lesions. To clarify the influence of genetic factors on KD susceptibility, Ferdosian et al. (21, 22) conducted a meta-analysis from IL-10 polymorphisms, TNF-α rs1800629, CASP3 rs72689236 and FCGR2A rs1801274 polymorphisms, respectively. The results showed that the IL-10-592 A > C polymorphism, CASPS rs72689236 and FCGR2A rs1801274 polymorphisms may mediate the susceptibility of individuals to KD. Another meta-analysis has also indicated that the CD32a polymorphism rs1801274 is strongly associated with KD pathogenesis and its A allele influences the incidence of KD (23).

The SARS-CoV-2 outbreak epidemic in 2019, with much clear evidence of its association with KD, has generated interest among researchers to link and further analyze both SARS-CoV-2 infection and KD. To clarify the clinical features and investigate the pathogenesis of pediatric multisystem inflammation syndrome (PMIS), Zou et al. (24) searched PubMed and Embase for relevant cases and performed a meta-analysis. This study found that PMIS usually had prolonged fever, gastrointestinal symptoms, cardiogenic shock and Kawasaki-like syndrome, and that SARS-CoV-2 infection was associated with a significant inflammatory state. Abrams et al. (25) published a systematic review with similar findings, which suggested that the vast majority of the 440 children with MIS included presented with gastrointestinal, skin/mucosal symptoms and cardiovascular symptoms, with significantly elevated levels of laboratory tests for inflammatory markers such as C-reactive protein, interleukin-6 and fibrinogen. Lamrani et al. (26) published a case series meta-analysis titled Kawasaki Disease Shock Syndrome vs. Classical Kawasaki Disease: a Meta-analysis and Comparison With SARS-CoV-2 Multisystem Inflammatory Syndrome in 2021 and first explored the differences between KD shock syndrome and traditional KD. This study found that children with KD shock syndrome were characterized by older age, higher inflammatory index C-reactive protein, higher odds of intravenous immunoglobulin resistance, longer hospital stay, and higher rate of coronary artery abnormalities compared to children with classical KD. In addition, it has also been suggested that SARS-Cov2 can act as a trigger that can lead to a second recurrence of KD in genetically susceptible individuals (27).

It can be found that the current KD research mainly focuses on the etiological mechanisms of the disease, therapeutic measures, and the relationship with SARS-CoV-2 infection. Although the above systematic reviews and meta-analyses synthesize some of the research findings in the field of KD research from different perspectives, they do not provide a more comprehensive picture of the current status of research in the field of KD and the changes in hot spots. Therefore, it has become an urgent scientific problem to conduct research on the current status and hot trends of KD as an object of inquiry.

Research objectives and expected contributions

Bibliometric analysis is a tool for analyzing published academic publications and the development trends and hot spots of a research field through data statistics (28). By analyzing indicators such as the number of citations, the author's work list, national or thematic bibliography, and publication mode, the leading direction of the research field and the institutions and scholars who have found the most research output can be determined (28, 29). There is no denying that bibliometric analysis is widely used in many different disciplines, including economics and medicine. KD has always been a disease that clinicians and scholars pay close attention to.

However, there has been no bibliometric analysis and summary of the research publications on KD in the past 5 years. Therefore, this study aims to provide a comprehensive understanding of the global trends in KD research over the past 5 years by using a bibliometric approach, using the Clarivate Analytics Web of Science (WOS) database and Elsevier's Scopus database as literature sources, to evaluate the current state of research and expect to identify current research hotspots and frontiers.

Method

Data collection

Electronic literature search was performed through the Wed of Science (WOS) and the Scopus database, respectively. The search strategies for the different databases are shown below: WOS: Topic = ("Kawasaki disease") OR Topic = ("Kawasaki

syndrome") OR Topic = ("Mucocutaneous Lymph Node Syndrome"); Scopus: ALL ("Kawasaki disease") OR ALL ("Kawasaki syndrome") OR ALL ("Mucocutaneous Lymph Node Syndrome"). The time range is set from January 1, 2017 to December 31, 2021. The literature search and data collection time were August 15, 2022. The document language is limited to English. All bibliometric data ultimately included in the analysis were recorded in plain text format and cited references were exported from WOS/Scopus. The export file contains all the extracted information about the type and main content of the study. The literature search was carried out by two authors independently, and then the results of the two were compared. If there were any differences, the authors discussed with the third independent author and selected the optimal results.

Data cleaning

The collected literature data were first imported into Citespace (version 6.1.R2), and the duplicate literature was removed, as well as the conference abstracts and non-research articles (including editorials, abstracts, letters, news and newsletters, etc.). Finally, 5,848 and 6,804 documents were obtained from WOS and Scopus, respectively.

Data analysis

Bibliometrix Biblioshiny R-Package software (https://bibliometrix.org/biblioshiny/biblioshiny1.html) is used to analyze bibliometric data and VOSviewer is used (version 1.6.18) visualize the relevant network relationships (30, 31). In addition, Microsoft Excel 2019 was used to draw graphs and make data tables. Bibliometric data, including title of research paper, number of citations, publication year, author identity, author's country, publishing institution and keywords, etc.

This study analyzed the number of papers published and the publication trend of this research topic in the past 5 years. The top journals with the highest number of published papers, the most cited journals and the top 5 most cited articles were analyzed. The most influential research institutions and countries were analyzed based on the highest number of papers published on the research topic during 2017–2021. The top 10 most influential and productive authors were also analyzed and the co-citation author network and author collaboration network were mapped, clustered as "Leading Eigenvalues," based on a higher "Betweenness." The *h*-index was selected to evaluate the scientific influence of research authors/literature journals (32). And h-index is generated through Bibliometrix Biblioshiny analysis of bibliometrics data. International cooperation in the top 10 countries for Kawasaki disease research was quantified using Multi-country Publications (MCP) and Single Country Publications (SCP) scales. In addition, VOSviewer generates a national collaboration network to visualize research collaborations between countries. The keywords of the literature on this research topic were analyzed and word cloud maps were drawn to visualize the weight of keywords plus. Also, a graph of three domains was constructed in order to observe the inflow and outflow between the top 12 authors, the top 15 authors keywords and the top 10 countries that contributed to Kawasaki disease research in the last 5 years.

Result

Search result

Initially, 7,032 and 8,661 documents were obtained from WOS and Scopus, respectively. According to the inclusion and exclusion criteria, after excluding 1,183 ineligible articles and 1 duplicate article from the WOS database search, 5,848 studies were retained, including 5,102 Articles (87.24%) and 746 Reviews (12.76%); after excluding 1,857 ineligible or duplicate documents, the Scopus database finally 6,804 studies were retained, which included 5,154 Articles (75.75%) and 1,650 Reviews (24.25%). All bibliometric information of the above screened literature was further analyzed.

Major characteristics of the included studies

WOS database

The 5,848 included documents originated from 1,793 different journals and were published by 56,890 authors (the average citation per document and per document per year were 2.62 and 13.82), of which 103 were single-author documents. The average growth rate of published articles per year is 19.83%. Japan was the country with the highest total citations (40.75%), followed by the United States (23.81%) and the United Kingdom (6.35%). On the other hand, the country with the highest average number of citations for papers was Switzerland (51.09).

Scopus database

The 6,804 included studies were derived from 2,124 journals and published by 32,762 authors (mean 7.43 authors per paper). Of these, 295 were single-author literature, authored independently by 245 authors. The average annual growth rate of published articles is 24.05%. In the Scopus database, the United States is the country with the highest total citations (21.63%), followed by China (10.17%) and Germany (5.98%), while Japan is in 9th place in the world with a citation rate of 3.96%. In addition, the country with the highest average citations was Germany with 44.89.

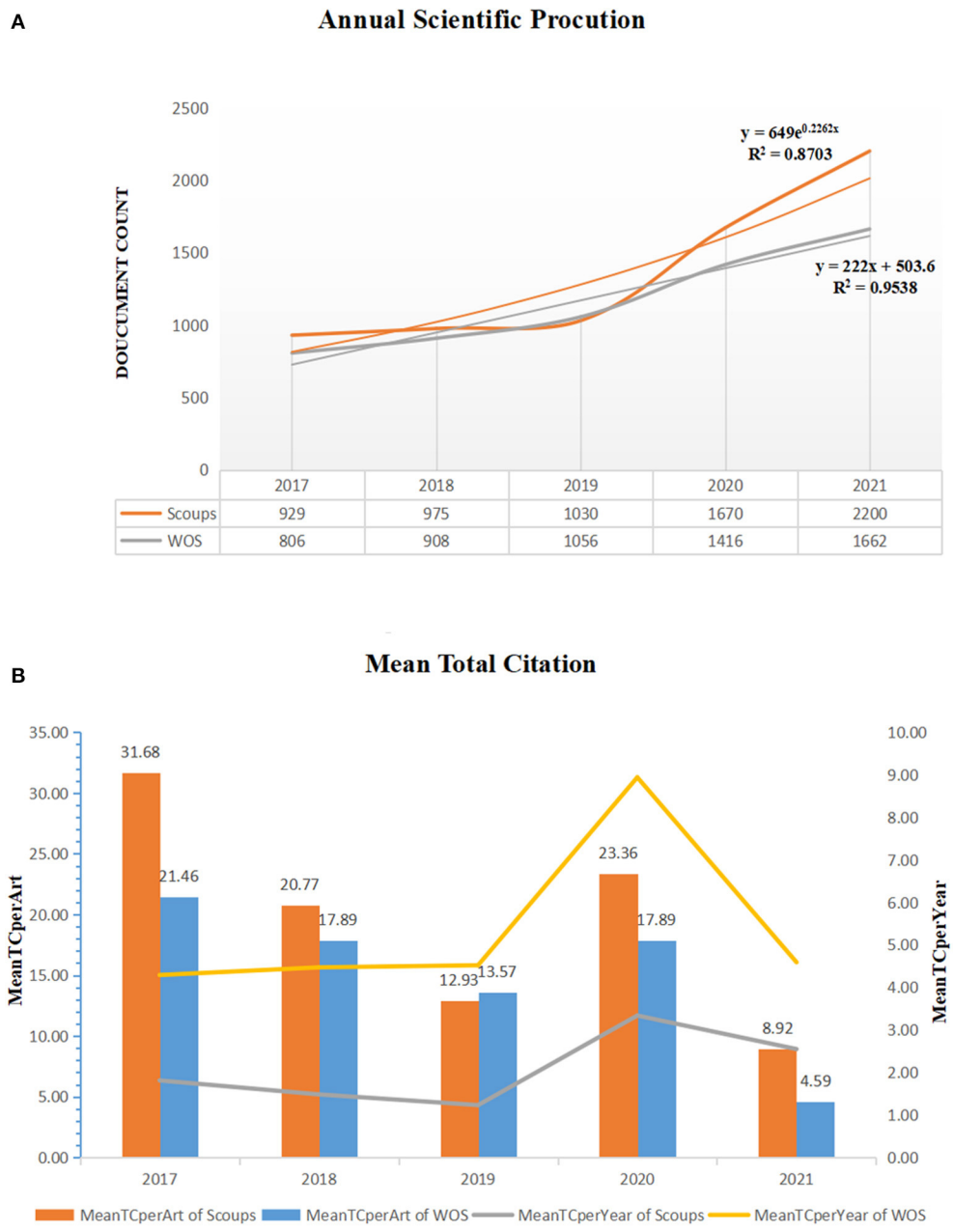

FIGURE 1
Global publication trends **(A)** and citation trends **(B)** for KD research. The left vertical coordinate indicates the average number of citations per document, and the right vertical coordinate indicates the average number of citations per year.

Global trend of publication and citation

In the last 5 years (2017–2021), t the number of publications and citations of KD research are continuing to grow, with slightly inconsistent growth trends for the two databases. The publication and citation trends for the WOS and Scopus databases are shown in Figure 1. Fitting curves between time and the number of published studies per year, where Scopus and WOS used exponential and linear functions, respectively. And their *R*-squared values were 87.03 and 95.38%, respectively. The average citations and average annual citations of studies published in the two databases from 2017 to 2021 are shown in Figure 1B. The average citations of the studies published in Scopus were higher than the average citations of WOS's; for the citation trend curve, both databases showed a significant increase in

TABLE 1 The top 15 journals with the largest number of published papers about KD (2017–2021).

Rank	WOS							Scopus						
	Sources	Country	NP (%), $n = 5,848$	TC	h-index	JCR®2022 impact factor	JCR®2022 category (quartile)	Sources	Country	NP (%), $n = 6,804$	TC	h-index	JCR®2022 impact factor	JCR®2022 category (quartile)
1	Scientific reports	England	146 (2.50%)	1,855	22	4.996	Multidisciplinary sciences (Q2)	Frontiers in pediatrics	Switzerland	142 (2.09%)	1,193	16	3.569	Pediatrics (Q2)
2	PLoS ONE	USA	120 (2.05%)	1,187	18	3.752	Multidisciplinary sciences (Q2)	Frontiers in immunology	Switzerland	124 (1.82%)	2,797	26	8.786	Immunology (Q1)
3	Frontiers in pediatrics	Switzerland	112 (1.91%)	855	15	3.569	Pediatrics (Q2)	Scientific reports	England	97 (1.43%)	1,140	18	4.996	Multidisciplinary sciences (Q2)
4	Pediatrics international	Japan	60 (1.02%)	479	10	1.617	Pediatrics (Q4)	Cardiology in the young	USA	78 (1.15%)	310	9	1.023	Pediatrics (Q4)
5	Cardiology in the young	USA	56 (0.95%)	190	8	1.023	Pediatrics (Q4)	PLoS ONE	USA	73 (1.07%)	904	18	3.752	Multidisciplinary sciences (Q2)
6	Journal of infection and chemotherapy	Japan	56 (0.95%)	316	10	2.065	Infectious diseases (Q4); pharmacology and pharmacy (Q4)	Journal of pediatrics	USA	66 (0.97%)	1,276	19	6.314	Pediatrics (Q1)
7	Circulation journal	Japan	53 (0.91%)	649	13	3.35	Cardiac and cardiovascular systems (Q3)	International journal of molecular sciences	Switzerland	62 (0.91%)	1,055	17	6.91	Chemistry (Q1)
8	Medicine	USA	52 (0.89%)	190	8	1.817	Medicine, general and internal (Q3)	Pediatric rheumatology	England	58 (0.85%)	464	10	3.413	Pediatrics (Q2)
9	Journal of pediatrics	USA	50 (0.85%)	779	15	6.314	Pediatrics (Q1)	Pediatric infectious disease journal	UAS	50 (0.73%)	1,176	13	3.806	Pediatrics (Q1); infectious diseases (Q3); immunology (Q3)
10	Clinical and experimental nephrology	Japan	44 (0.75%)	354	10	2.617	Urology and nephrology (Q3)	Pediatrics international	Japan	50 (0.73%)	462	9	1.617	Pediatrics (Q4)
11	Pediatric rheumatology	England	44 (0.75%)	322	9	3.413	Pediatrics (Q2); rheumatology (Q3)	BMJ case reports	Japan	47 (0.69%)	180	6	N/A	N/A

(Continued)

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TABLE 1 (Continued)

Rank	WOS							Scopus						
	Sources	Country	NP (%), $n = 5,848$	TC	h-Index	JCR®2022 impact factor	JCR®2022 category (quartile)	Sources	Country	NP (%), $n = 6,804$	TC	h-Index	JCR®2022 impact factor	JCR®2022 category (quartile)
12	Pediatric infectious disease journal	UAS	42 (0.71%)	423	11	3.806	Pediatrics (Q1); infectious diseases (Q3); infectious diseases (Q3);	BMC pediatrics	England	45 (0.66%)	317	11	2.567	Pediatrics (Q3)
13	Internal medicine	Japan	41 (0.70%)	142	6	1.282	Medicine, general and internal (Q4)	Medicine	USA	44 (0.65%)	865	14	1.817	Medicine, general and internal (Q3)
14	Frontiers in immunology	Switzerland	40 (0.68%)	694	13	8.786	Immunology (Q1)	International journal of rheumatic diseases	Australia	42 (0.62%)	488	9	2.558	Rheumatology (Q4)
15	International journal of molecular sciences	USA	39 (0.66%)	361	11	6.208	Biochemistry and molecular biology (Q1); chemistry, multidisciplinary (Q2)	Clinical rheumatology	England	41 (0.85%)	614	14	3.65	Rheumatology (Q3)

NP, number of papers; TC, total citation; JCR, journal citation report.

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citations of documents in 2019–2020, followed by a drop in both.

Most productive journals, most cited journals and journal publishing trends

When analyzing the total number of publications for the KD research, WOS had 2.50% of the papers published in *Scientific Reports*, followed by *PLoS ONE* (2.05%) and *Frontiers in Pediatrics* (1.91%), while Scopus had Frontiers in Pediatrics published the most papers (2.09%), followed by Frontiers in Immunology (1.82%) (as shown in Table 1). It is worth noting that the vast majority of the top 15 journals in terms of the number of published papers are not high impact factor journals, nor are they based on 2022 JCR® journals in the Q2 category or higher. Figure 2 shows the publication trend for the top 6 journals in both databases over the last 5 years, with *Frontiers in Pediatrics* showing the most significant growth in both databases. For the WOS database, Scientific Reports is the second fastest growing journal, while the second fastest growing journal in Scopus is Frontiers in Immunology.

Most influential authors and their collaborations networks

The top 10 most influential authors in the two databases varied widely. As shown in Table 2, Goodman SG, Kazunori Kataoka, Kimura Takeshi, and Marx Nikolaus had the highest influence in KD research in WOS using the *h*-index as a reference standard; while in Scopus, Wang Y, Burns JC and Newburger JW were the most influential. As for the number of published studies, (i) Shibagaki Yugo (NP = 70), (ii) Kuo Ho-Chang (NP = 61) and (iii) Burns JC (NP = 51) were the most contributing authors in the WOS database; however, in the Scopus database, (i) Wang Y (NP = 111), (ii) Zhang Y (NP = 95) and (iii) Li Y (n = 83) have the highest number of published studies.

Looking at the network of co-cited authors, the most co-cited authors in WOS were Newburger JW [link (L) = 19, link strength (LS) = 7,617, citation(C) = 1,241] and Mccrindle (L = 19, LS = 5,409, C = 1,114), while in Scopus, it was Burns JC (L = 20, LS = 40,839, C = 3,031) and Newburger JW (L = 20, LS = 34,633, C = 2,845) (Figures 3A, B). Among the retrieved WOS documents, the most important collaborations among researchers were Hoshino Junichi (L = 17, LS = 254, C = 185) and Ubara Yoshifumi (L = 16, LS = 255, C = 186). For the Scopus files, Wang L (L = 17, LS = 107, C = 1,538), Zhang L (L = 15, LS = 92, C = 935), and Zhang Y (L = 17, LS = 83, C = 1,234) were the most collaborative authors (Figures 3C, D).

Most productive institutions and their cooperation

The most productive institutions for KD research are shown in Table 3. For WOS files, three Japanese institutions, St. Marianna University, Kawasaki medical school, and The University of Tokyo, were the most productive institutions; while for Scopus, Harvard Medical School, University of California, and Tehran University of Medical Sciences were in the top three. Analysis of institutional collaboration revealed that St. Marianna University (L = 19, LS = 713, C = 6,591), Kawasaki Medical School (L = 19, LS = 657, C = 5,746), and The University of Tokyo (L = 19, LS = 643, C = 6,102) were the most collaborative institutions in the WOS (Figure 4A). While in Scopus, Harvard Medical School (L = 19, LS = 3,189, C = 3,266), Kaohsiung Chang Gung Memorial Hospital (L = 19, LS = 2,801, C = 327) and Boston Children's Hospital (L = 19, LS = 2,644, C = 272) were the most collaborative institutions (Figure 4B).

World research production and collaboration

Table 4 shows the top ten major countries publishing on the topic of KD and their cooperative publishing proportion. In both databases, we can find that Japan, the United States and China are the countries with the highest number of published studies. For WOS, Japan leads in the number of both SCP and MCP. Interestingly, Canada has the highest rate of MCP (MCP Ratio = 0.562), followed by the UK (MCP Ratio = 0.556) and France (MCP Ratio = 0.507). While in Scopus, China was in first place in both SCP and MCP, and the highest percentage of highest rate of MCP was in the UK (MCP Ratio = 0.456).

Figure 5 shows the network of collaborations among top 50 countries of KD research. And the top 50 countries could be roughly divided into several clusters using the "association strength" clustering method. For the WOS literature, these countries were broadly grouped into 4 clusters, with Japan (LS = 1,726, blue cluster), the USA (LS = 1,611, green cluster), and the UK (LS = 815, red cluster) being the most collaborative, while in Scopus, these countries were grouped into 5 clusters, suggesting that USA (LS = 1,157, green cluster), UK (LS = 723, red cluster), and Germany (LS = 430, yellow cluster) were the most critical in terms of KD research collaboration.

Most cited literature

When analyzing the literature from WOS, the average citations per document and per document per year were 2.62 and 13.82, respectively; while the average citations per

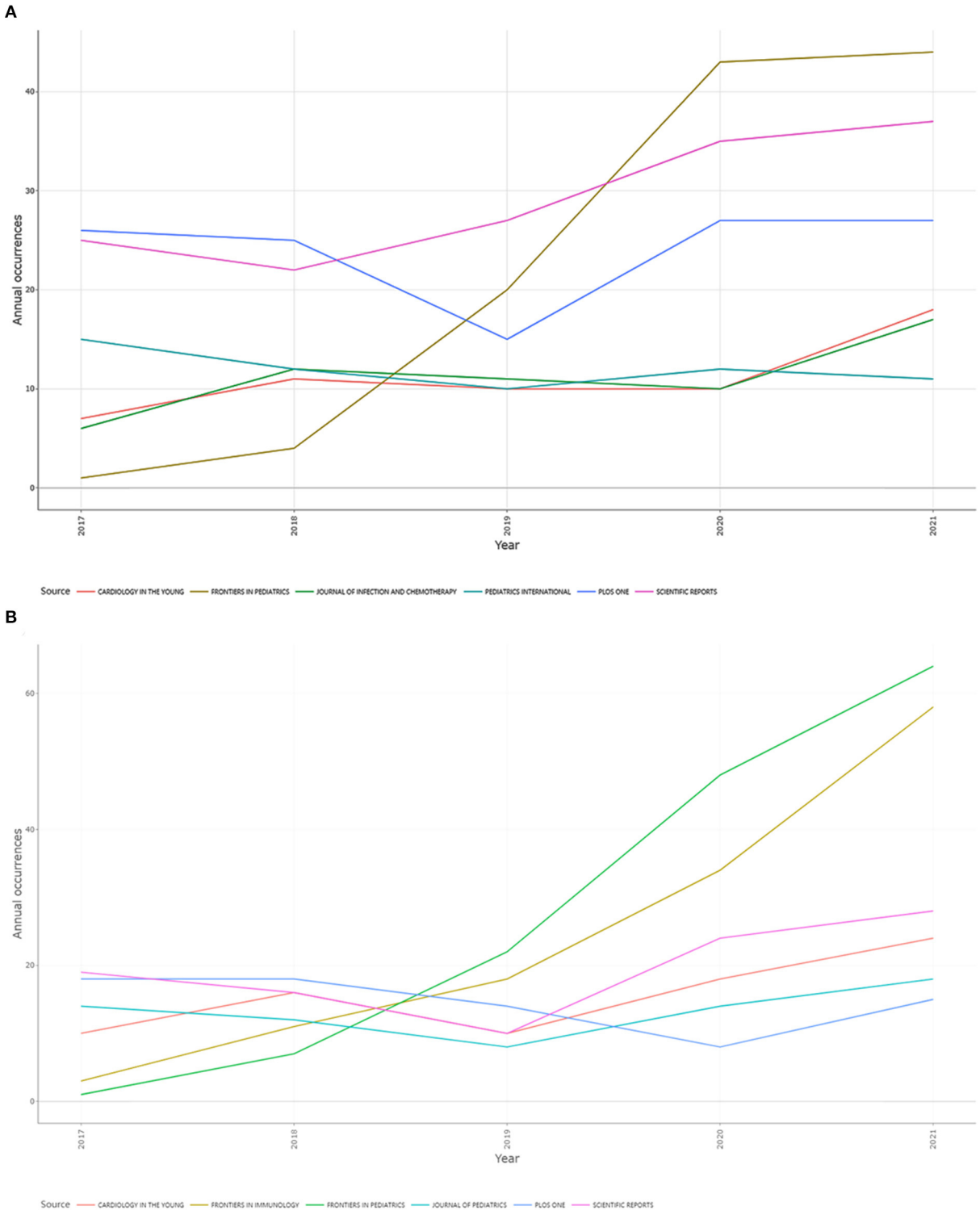

FIGURE 2
A line chart showing the annual trend of documents published in the top six journals of KD research (2017–2021): **(A)** WOS and **(B)** Scopus. The abscissa represents different years, the ordinate represents the number of documents published each year, and different color curves represent different journals.

TABLE 2 The h-index, total citations and numbers of published papers of the top 10 most influential and productive authors.

WOS database								Scopus database							
Based on the h-index				Based on the number of published papers				Based on the h-index				Based on the number of published papers			
Authors	h-index	TC	NP	Authors	h-index	TC	NP	Authors	h-index	TC	NP	Authors	h-index	TC	NP
Goodman SG	21	4,102	24	Shibagaki Yugo	14	746	70	Wang Y	20	1,617	111	Wang Y	20	1,617	111
Kataoka Kazunori	21	1,561	41	Kuo Ho-Chang	13	608	61	Burns JC	19	3,755	57	Zhang Y	18	1,383	95
Kimura Takeshi	21	2,744	33	Burns JC	18	3,194	51	Newburger JW	18	4,208	40	Li Y	14	815	83
Marx Nikolaus	21	4,672	23	Kashihara Naoki	15	1,193	46	Zhang Y	18	1,383	95	Kuo Ho-Chang	16	843	77
Lopes RD	20	5,105	21	Kataoka Kazunori	21	1,561	41	Arditi M	17	714	25	Zhang L	16	1,001	74
Kiss RG	19	3,938	21	Tremoulet AH	15	999	39	Tremoulet AH	17	1,214	45	Wang X	15	1,068	67
Burns JC	18	3,194	51	Yasuda Satoshi	10	263	38	Kuo Ho-Chang	16	843	77	Li X	14	636	66
Hagstrom Emil	18	3,844	20	Yamagata Kunihiro	15	721	36	Zhang L	16	1,001	74	Zhang J	15	785	65
Liberopoulos Evangelos	18	3,844	20	Kozuma Ken	13	2,095	36	Cimaz R	15	682	23	Liu Y	14	690	63
Newburger JW	17	3,629	34	Newburger JW	17	3,629	34	Wang X	15	1,068	67	Burns JC	19	3,755	57

TC, total citation; NP, number of published papers.

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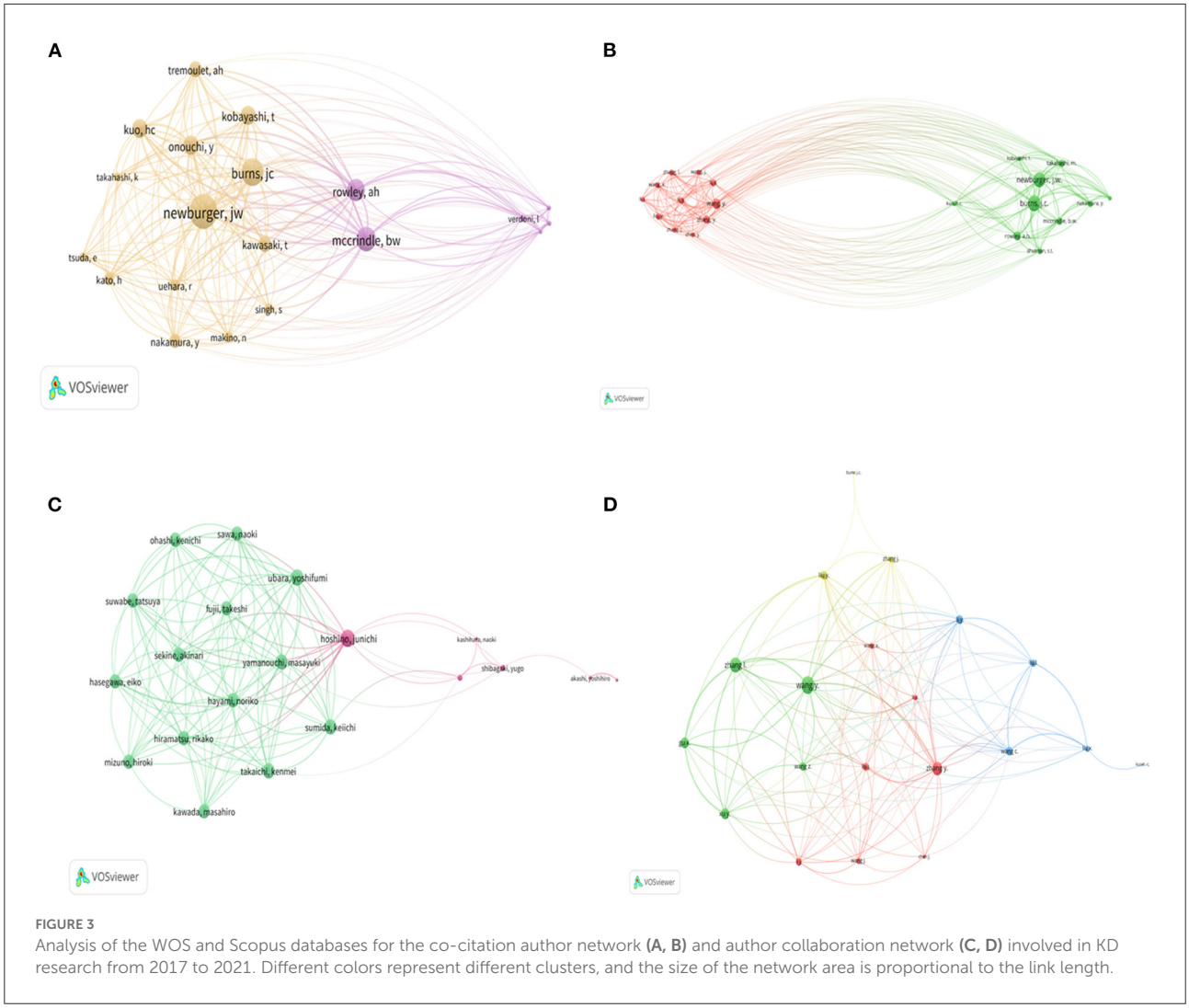

FIGURE 3
Analysis of the WOS and Scopus databases for the co-citation author network **(A, B)** and author collaboration network **(C, D)** involved in KD research from 2017 to 2021. Different colors represent different clusters, and the size of the network area is proportional to the link length.

TABLE 3 Ten most productive affiliation in KD research in WOS and Scopus.

Rank	WOS database		Scopus database	
	Affiliation	Articles	Affiliation	Articles
1	St Marianna University School of Medicine	1,048	Harvard Medical School	177
2	Kawasaki Medical School	786	University of California	134
3	The University of Tokyo	753	Tehran University of Medical Sciences	128
4	Keio University	551	University of Toronto	120
5	Osaka University	531	China Medical University	105
6	Juntendo University	393	Capital Medical University	93
7	Kyoto University	384	Huazhong University of Science and Technology	91
8	Nippon Medical School	382	Chang Gung University College of Medicine	85
9	University of Tsukuba	329	Guangzhou Medical University	80
10	Yokohama-City University	328	Baylor College of Medicine	77

TABLE 4 The top 10 major publishing countries with KD research and their cooperative publishing proportion.

Rank	WOS database				Scopus database			
	Country	NP (%), $n = 5,848$	SCP	MCP	Country	NP (%), $n = 6,804$	SCP	MCP
1	Japan	3,356 (57.4%)	2,961	395	China	1,350 (19.84%)	1,212	138
2	USA	631 (10.8%)	390	241	Japan	1,237 (18.18%)	1,094	134
3	China	563 (9.6%)	496	67	USA	1,101 (16.18%)	887	214
4	India	122 (2.1%)	109	13	Italy	332 (4.88%)	273	59
5	Italy	120 (2.1%)	92	28	India	226 (3.31%)	202	24
6	United Kingdom	108 (1.8%)	48	60	Korea	215 (3.16%)	193	22
7	Korea	101 (1.7%)	89	12	Turkey	200 (2.94%)	192	8
8	Canada	89 (1.5%)	39	50	United Kingdom	180 (2.65%)	98	82
9	Turkey	74 (1.3%)	69	5	Iran	175 (2.57%)	140	35
10	France	71 (1.2%)	35	36	Germany	161 (2.37%)	116	45

NP, total numbers of published paper; SCP, single country publication; MCP, multiple countries publication.

document and per document per year for the Scopus literature were 2.52 and 17.87, respectively. Among them, McCrindle et al. (5) entitled "Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: a Scientific Statement for Health Professionals From the American Heart Association," with 1,400 total citations, was ranked first in WOS and was published in Circulation. And it was affiliated with American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young. While in Scopus, the top total citation is the review article entitled" Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association" by Benjamin et al. (33) published in Circulation. The article is part of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee and has been cited a total of 6,057 times. The top 5 most cited documents on the topic of KD in the WOS and Scopus databases are shown in Table 5, and all of these studies have been cited more than 1,000 times.

Literature survey of top 5 cited documents to KD research in WOS and Scopus database

Since the American Heart Association (AHA) published the first edition of its guidelines for the diagnosis, treatment, and long-term management of KD in 2004, more refined versions have been updated (5). McCrindle et al. (5) on behalf of the AHA, based on a review of the 2004 AHA scientific statement as well as the most recent version of the guidelines, with a focus on evaluating the most recent published studies at that time, the guidelines were revisions as necessary. The guidelines provide a new discussion of the epidemiology, genetics, pathogenesis, pathology, natural history, and long-term prognosis of KD, providing clinicians with new recommendations for the diagnosis, acute phase treatment, and long-term management of KD. However, the contributing expert group noted that there is still a lack of preventive measures for KD, while a small percentage of children with standardized treatment still develop coronary artery lesions (5).

In the same year, Benjamin et al. (33) published the 2017 edition of the AHA Heart Disease and Stroke Statistics Report. Since 2006, the AHA has included heart disease, stroke, and seven life factors associated for maintaining a healthy heart in its statistics and updated the report annually. Each revision of the Report will incorporate the most current and representative US health data, add updated scientific insights, and new sections. In section 16 of this updated Report, the findings of recent studies on KD were summarized, including the diagnosis of the disease, etiology and genetic mechanisms, and epidemiological data based on country, geography, and ethnicity. In addition, it identified late disease diagnosis, age at onset <6 months, male gender, and Asian population as risk factors for the development of coronary artery lesions (33). The following year, the authors conducted a revision of the 2018 version. Based on the content of the old version, a new statement was added to section 14 of the report: as an acute inflammatory disease of acquired origin, KD is most common in East Asian populations (including Japan, Taiwan, and Korea), and its incidence is increasing year by year (37). Since then, Virani et al. also completed an update of the data for that year in 2020. This version added data on KD cases from the US mainland. USA had 6,000 KD discharges in 2016 (4,000 men and 2,000 women); and KD contributed to 5 patients' underlying mortality and 10 patients' all-cause mortality, respectively, in the 2017 US mortality data (38). Notably, the Statistical Update has been widely cited due to its scientific validity and completeness, with a total of more than

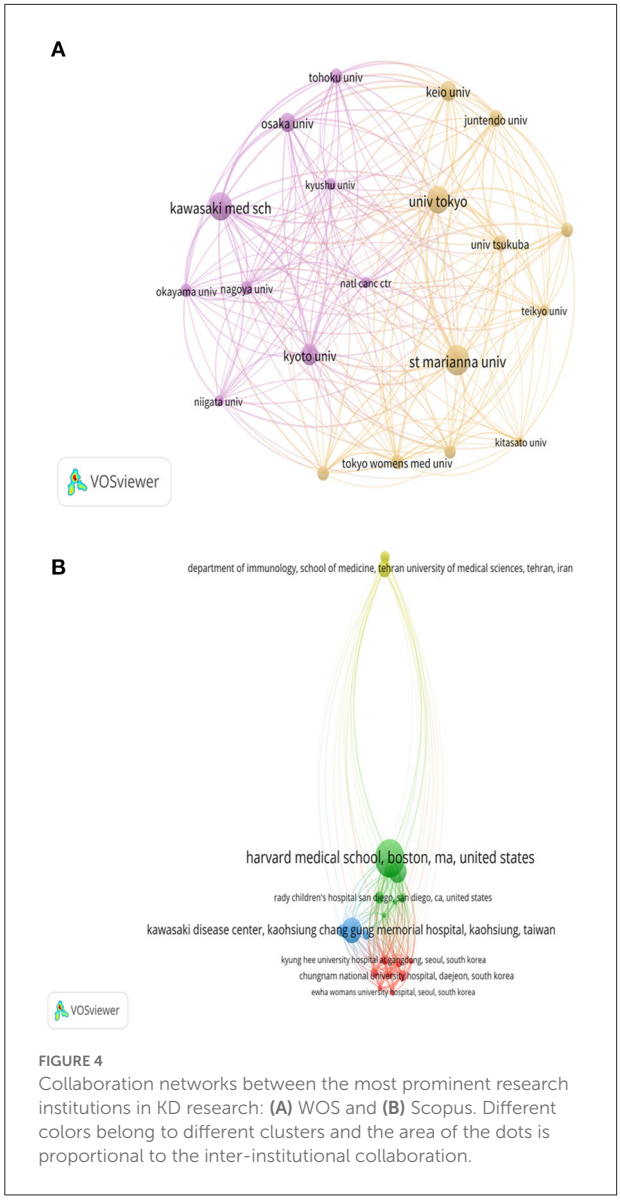

FIGURE 4
Collaboration networks between the most prominent research institutions in KD research: **(A)** WOS and **(B)** Scopus. Different colors belong to different clusters and the area of the dots is proportional to the inter-institutional collaboration.

20,000 citations, and in the first 7 months of 2017 alone, the 2017 Statistical Update has been accessed more than 106,500 times (33, 37).

Since the outbreak of the novel coronavirus SARS-CoV-2, several national health agencies have been involved in monitoring and analyzing the outbreak data. The Garg et al. (34) application COVID-19-Associated Hospitalization Surveillance Network (COVID-NET), led by the CDC COVID-NET team, conducted population-based disease data surveillance. The report included data on disease in patients hospitalized for COVID-19 in March 2020, and the analysis noted the highest prevalence in older adults aged ≥65 years, while the vast majority of hospitalized patients (~90%) had underlying disease, with chronic conditions such as obesity, hypertension, diabetes, and cardiovascular disease being prevalent (34).

Gupta et al. (36) conducted a comprehensive review of the pathophysiological and clinical effects of COVID-19 on various organ systems using the extra-pulmonary manifestations of SARS-CoV-2 as the object of analysis. This review summarizes the viral pathophysiology and virulence mechanisms of SARS-CoV-2, and pioneers the possible clinical manifestations and disease mechanisms in multiple extra-pulmonary organ systems (including hematological, cardiovascular, renal, gastrointestinal, hepatobiliary, endocrine, ocular and neurological, and skin) and special disease conditions in humans, including children and pregnant women, separately. In the section on pediatric-associated SARS-CoV-2 infection, this review summarized the definition of MIS-C and potentially useful therapeutic measures and suggest that the underlying mechanism in most children with MIS-C may arise from an acquired immune response in the body rather than direct SARS-CoV-2 injury (36).

To assess the incidence and clinical characteristics of patients with KD-like illness diagnosed during the SARS-CoV-2 epidemic, a cohort study was conducted by Verdoni et al. (6) in Bergamo Province, Italy. The study reviewed information on the disease in children who met the diagnostic criteria for KD from January 1, 2015 to April 20, 2020, and grouped these children according to the timing of the local SARS-CoV-2 outbreak. Ultimately, it was found that the incidence of KD in the area increased dramatically by 30-fold after the virus epidemic, and most of the children diagnosed showed positive results for SARS-CoV-2. These children who tested positive tended to be relatively older, had a higher incidence of cardiac complications, and had more severe disease manifestations (6). Although this is a small sample case series study, it has been widely cited because it explores the potential relationship between the surge of Kawasaki-like disease and the SARS-CoV-2 epidemic, which has far-reaching clinical implications. Meanwhile, Feldstein et al. (35) summarized the epidemiological and clinical characteristics of a total of 186 children with MIS in 26 states in the United States and indicated that SARS-CoV-2-associated MIS-C can be life-threatening in a population of healthy minors. This study found that ~40% of the 186 children presented with Kawasaki-like clinical features, and 80% of children with MIS were placed in intensive care. In terms of pharmacological treatment, nearly half of the children required vasoactive drugs and immunomodulators were commonly applied to fight multisystem inflammation.

Keywords analysis and topic trends

Text analysis revealed that the WOS documents had 10,458 keywords plus and 11,469 author keywords, while the Scopus documents had 25,423 keywords plus and 11,078 author keywords. The direction and themes of KD research

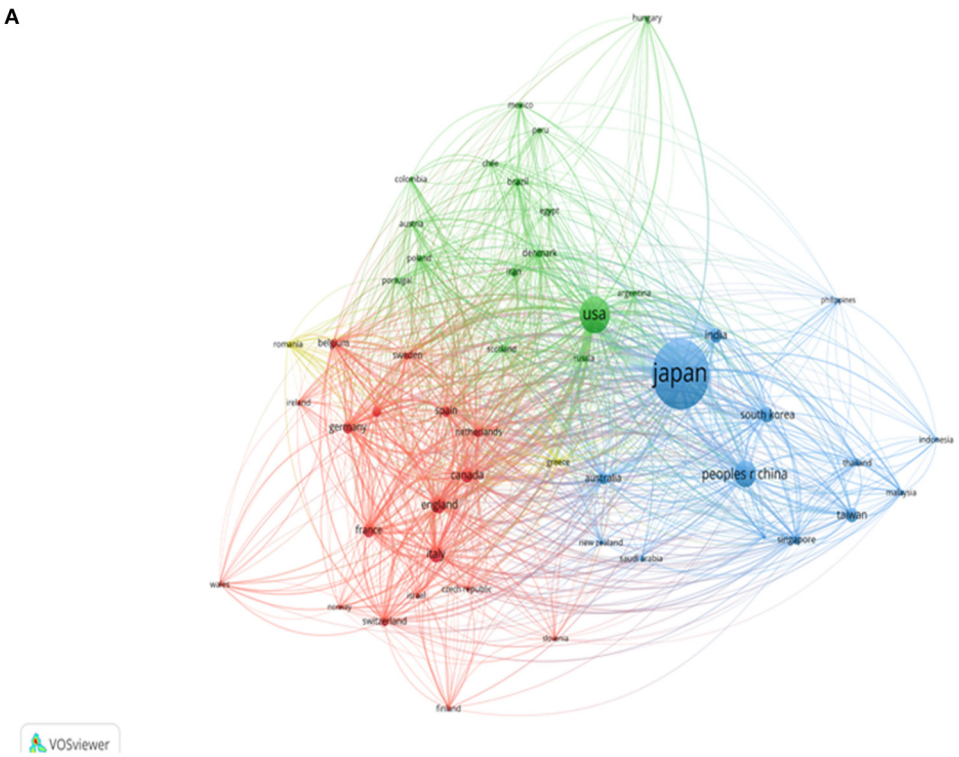

FIGURE 5
Network visualization of collaborations among countries of KD research in WOS **(A)** and Scopus **(B)**.

TABLE 5 The top 5 most cited documents on KD (2017–2021).

Rank	References	Title name	Document type	Journal name	DOI	Total citations
WOS database						
1	Mccrindle et al. (5)	Diagnosis, treatment, and long-term management of kawasaki disease: a scientific statement for health professionals from the american heart association	Review article	Circulation	10.1161/CIR.0000000000000484	1,400
2	Garg et al. (34)	Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019—COVID-net, 14 states, march 1-30, 2020	Research article	Mmwr-morbidity and mortality weekly report	10.15585/mmwr.mm6915e3	1328
3	Verdoni et al. (6)	An outbreak of severe Kawasaki-like disease at the Italian epicenter of the SARS-CoV-2 epidemic: an observational cohort study	Research article	The Lancet	10.1016/S0140-6736(20)31103-X	1244
4	Feldstein et al. (35)	Multisystem inflammatory syndrome in U.S. children and adolescents	Research article	The new England journal of medicine	10.1056/NEJMoa2021680	1158
5	Gupta et al. (36)	Extrapulmonary manifestations of COVID-19	Review article	Nature medicine	10.1038/s41591-020-0968-3	1127
Scopus database						
1	Benjamin et al. (33)	Heart disease and stroke statistics-2017 update: a report from the American heart association	Review article	Circulation	10.1161/CIR.0000000000000485	6057
2	Benjamin et al. (37)	Heart disease and stroke statistics-2018 update: a report from the American heart association	Review article	Circulation	10.1161/CIR.0000000000000558	4240
3	Virani et al. (38)	Heart disease and stroke statistics-2020 update: a report from the American heart association	Review article	Circulation	10.1161/CIR.0000000000000757	3547
4	Mccrindle et al. (5)	Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American heart association	Review article	Circulation	10.1161/CIR.0000000000000484	1647
5	Verdoni et al. (6)	An outbreak of severe Kawasaki-like disease at the Italian epicenter of the SARS-CoV-2 epidemic: an observational cohort study	Research article	The Lancet	10.1016/S0140-6736(20)31103-X	1360

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FIGURE 6
Visualized word-clouds of keywords plus in WOS **(A)** and Scopus **(B)** (Only show the first 50 keywords).

can be identified to further understand the current trend of the discipline by analyzing the keyword distribution. It is worth noting that keywords plus are essential words for exploring a scientific field and they often appear in the title of the article reference rather than in the title of the article (39). Supplementary Table S1 shows the top 10 most frequently occurring keywords plus and author keywords. Since this topic is aimed at KD research, the author keywords appeared most frequently with "Kawasaki disease," followed by "COVID-19," "SARS-CoV-2" and "children." In addition, we can find the top 10 author keywords mainly related to novel coronaviruses, disease mechanisms (vascular, inflammation, and mis-c) and their therapeutic measures (intravenous, immunoglobulin). As for keywords plus, it is mainly related to the diagnosis and management of the disease (Figure 6). The size and centering of the keyword in the Word Cloud reflects its frequency and magnitude.

By applying VOSviewer, the frequency of occurrence of keywords and the process of change over time were visualized. The top 50 author keywords with a frequency of at least 5 occurrences were analyzed for both databases (Figure 7).When the minimum cluster size was set to 10, keywords in the WOS literature were classified into four categories: (1) Novel coronavirus-associated multisystem inflammatory syndrome in children; (2) cardiac, renal, and vascular-related prognosis of KD; (3) symptoms of KD; and (4) diagnosis and treatment of KD; while keywords in the Scopus literature were classified into three categories: (1) Novel coronavirus-associated multisystem inflammatory syndrome in children associated multisystem inflammatory syndrome; (2) diagnosis, mechanism, and treatment of KD; and (3) cardiac-related complications of KD (Supplementary Table S2).The purple author keywords in Figure 7 appear earliest, while the yellow author keywords appear latest. The keywords "COVID-19," "multisystem inflammatory syndrome," and "pandemic" are the most recent in both data. Therefore, we can use the latest emerging keywords to understand the frontier hot spots of KD research.

Sankey diagrams: Three field plots on KD research

The Biblioshiny three-field plot represents the communication relationships between elements by combining rectangles representing different elements and connecting lines between rectangles, and is often used to visualize the connections between literature sources, countries/regions, affiliations, keywords, primary authors, cited sources, and author keywords. Where the larger the rectangle, the more communication between multiple components (40). Figure 8 shows a schematic representation of studies in the KD research literature on the relationship between authors (left), keywords (center), and authors' countries (right). The analysis identified which keywords of the KD study were most frequently used by different authors and countries. The analysis of the most frequently used keywords, source authors and countries suggested that five authors Kuo HC, Tremoulet AH, Singh Surjit, Burns JC, Newburger JW, five keywords, namely "Kawasaki disease," "covid-19," "SARS-CoV-2," "Japan," and "coronary artery disease," which originated from four countries, USA, Japan, UK, and Germany.

Discussion

Using bibliometric analysis methods and visualization software as technical support, this study explored the research trends and hotspots in the field of KD from 2017 to 2021. The number of papers published in this research area has continued to increase in the last 5 years, and the type is dominated by Article. Taking 2019, the year when the novel coronavirus outbreak epidemic started, as the boundary, the number of KD papers published grew even faster, and the growth characteristics of papers in different databases were not fully consistent, which shows that this research field may still have more scientific output in the coming years. The citation volume curve showed that the average annual citation volume was essentially flat during 2017–2019, while a significant fluctuation was observed

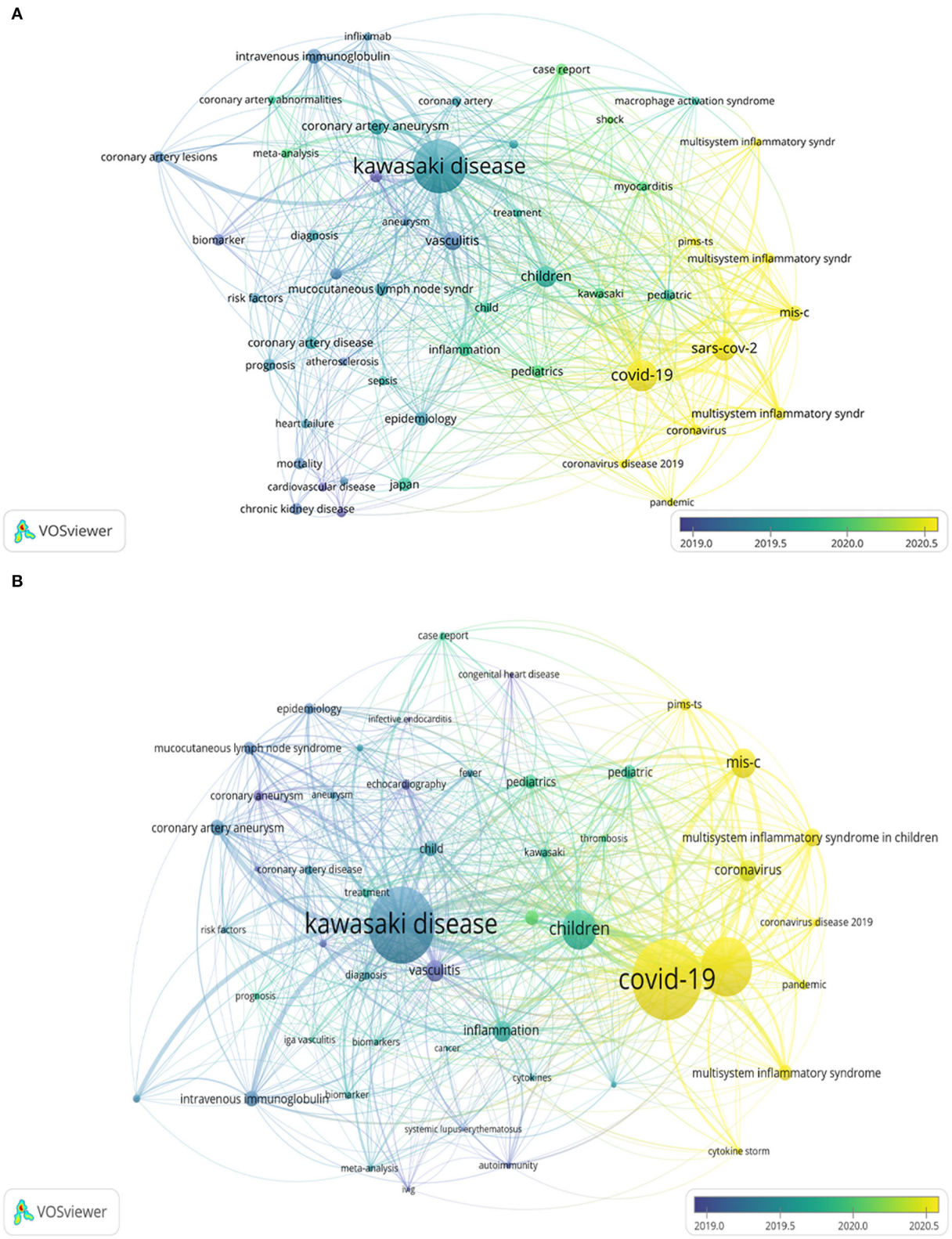

FIGURE 7
Time-based visualization of keywords variation: **(A)** WOS and **(B)** Scopus.

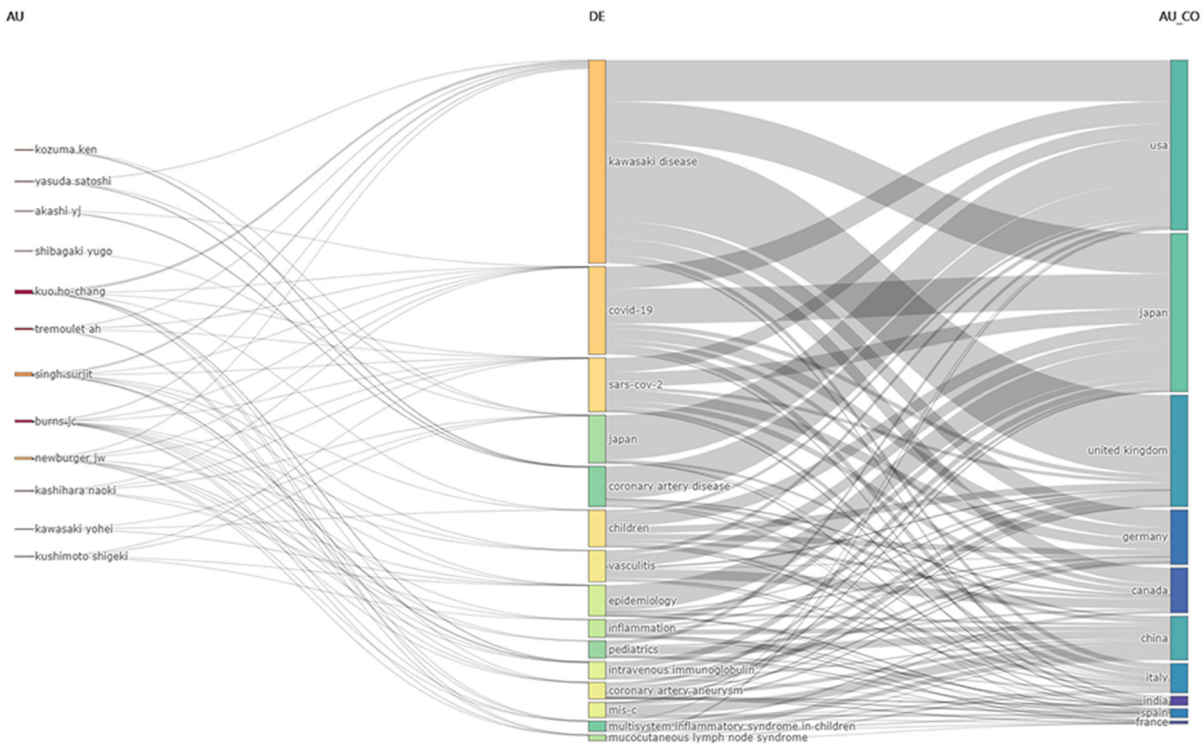

FIGURE 8
Three field plots on KD research. The picture represents the authors who have made the greatest contribution to KD research in the past 5 years (2017–2021), the author keywords and the inflow and outflow of countries. AU, author; DE, author Keywords; AU_CO, countries.

in 2020. The apparent increase in citation volume is consistent with the findings of another Google trend study on COVID-19 and Kawasaki disease (41). This may be related to the continued discovery of symptoms similar to Kawasaki disease following the COVID-19 outbreak (41, 42).

This study found that both WOS and Scopus database publications on KD research originated mainly three countries, Japan, USA, and China, with Japan and USA having the highest total number of citations in the WOS and Scopus databases, respectively. This result is not surprising, as Japan was the first country to identify and intensively study KD (43). KD has a high prevalence throughout Northeast Asia (38). In China, the overall prevalence of KD appears to be on the rise, with data from questionnaire surveys in Beijing and Shanghai in 2017 showing a prevalence of 46.3–55.1 per 100,000 Chinese children under 5 years of age, and 28.58–60.08 per 100,000 children younger than 5 years of age in Taiwan (44, 45). This has gradually drawn attention to KD in this populous country (46). In China, the first expert consensus was written by the Shaanxi Provincial Diagnosis and Treatment Center of Kawasaki Disease in 2021 to regulate the use of IVIG in KD (47). The above reasons have led to more in-depth research in these countries.

Of the top 10 most productive countries, 70% were developed countries, which may be related to the fact that these countries have access to more adequate research fund support. When analyzing national collaboration networks, KD research is most collaborative in developed countries such as Japan, USA, and the United Kingdom, reflecting the central position of these countries in the field of KD. However, none of the African countries have been included in these top productivity rankings. A study showed a map of the global incidence of KD, with African regions represented as gray areas due to lack of available data (48). However, a study from the African country of Egypt showed that missed undiagnosed or untreated KD may be prevalent in that country (49). The lack of awareness of KD in African countries and the poor surveillance mechanisms in government health institutions may have contributed to this result. There is a need for us to provide the necessary financial support and assistance to African countries in the surveillance and treatment of KD.

In the WOS database, the most cited article was written by McCrindle et al. (5); this was written to revise previous American Heart Association guidelines. This article provides more scientific and novel guidelines for the diagnosis and treatment of KD and highlights that individualized treatment

plans should be developed for different patients (5). In the Scopus database, "Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association" by Benjamin et al. (33) is the most cited. Analysis of the most cited literature from both databases shows that KD has been of great interest in the field of pediatrics, especially in terms of diagnosis, etiologic mechanisms and treatment of the disease. Since the COVID-19 epidemic, SARS-CoV-2 infection has been implicated as an infectious factor contributing to the dramatic increase in KD. Exploring the pathological mechanisms, clinical features and therapeutic measures of Kawasaki-like diseases associated with SARS-CoV-2 infection has become a hot research topic in recent years.

When evaluating the most productive journals based on number of publications, ie was found that not all of the top 15 journals in both databases were Q2+ journals base on 2022JCR ® and not all had high impact factors. For the WOS database, Scientific Reports ranked first in terms of number of publications, total citations and h-index, while in the Scopus database, Frontiers in Pediatrics was the highest contributor. Frontiers in Pediatrics has the fastest growth rate in the number of published papers in the last 5 years. However, analysis of the main source journals of cited literature reveals that most of the cited literature on KD research originates from high impact factor journals, such as *Circulation*, *The New England Journal of Medicine*, and *Lancet*. We speculate that it is more difficult to publish KD research papers in high impact factor journals, but they can receive more attention and citations than publishing them in low impact factor journals (50). And, in fact, an essential and important factor in order for an individual paper to obtain more citations is the effort put into writing the paper (51). Therefore, we believe that the quality of a research paper cannot be judged solely on the basis of the impact factor of the journal.

Based on the h-index and the number of papers published, this study analyzed the most influential and most productive authors, respectively. In addition, the most cited authors from the co-cited author network observed for both databases were Newburger JW and McCrindle BW and Burns JC, suggesting that their research results are widely followed and recognized. The author collaboration network shows that the author team with two authors, Hoshino Junichi and Ubara Yoshifumi, as the main leaders and the research teams led by Wang L, Zhang L and Zhang Y, respectively, are the most closely collaborated. The above information will help future researchers working in the field of KD research to more accurately and quickly identify influential research teams and to consult relevant individuals in a targeted manner.

In the WOS database, the top three most productive institutions were all from Japanese institutions and showed the highest level of collaboration and formed collaborative networks with other institutions. For the Scopus database, it is the US institutions that have the highest output, and the inter-institutional collaboration tips are dominated by US institutions. This implies that in the field of KD, two developed country research institutions, Japan and the United States, hold more research resources and are in the leading position in international research. And the analysis of global inter-country collaboration showed that Japan, the United States and the United Kingdom were the most cooperative countries.

Keyword analysis reveals the most common author keywords and the temporal sequence of keyword occurrences. "Kawasaki disease," "COVID-19," "SARS-CoV-2," and "children" were the most common keywords. Time-based keyword change analysis showed that the keywords "COVID-19," "multisystem inflammatory syndrome," and "pandemic" have appeared only recently. KD mainly causes systemic vascular inflammation mainly in infants and children (30). Our results showed a high frequency of the keyword "children," and the papers included in the analysis were mainly from the pediatric population. In the United States, the Centers for Disease Control proposed in May 2020 a new disease, MIS-C, which is associated with COVID-19 (52).These children share common features with KD, i.e., mild pulmonary signs but marked and severe systemic inflammation (53). The high frequency of "Kawasaki disease" and "COVID-19/SARS-CoV-2" also suggests an important association between Kawasaki disease and COVID-19. SARS-CoV-2 may be a factor in triggering Kawasaki disease (54). Such children with SARS-CoV-2-induced KD have an older age of onset and are characterized clinically by the development of myocarditis (26). Verdoni et al. (32) also revealed that Italian patients with COVID-19 can develop a more severe symptomatic Kawasaki-like disease, and these patients often require adjuvant glucocorticoid therapy. By analyzing the co-linear relationships of the keywords, we classified the authors' keywords. Notably, the keywords in both databases can be broadly classified into the categories of novel coronavirus-associated multisystem inflammatory syndrome in children, diagnosis and treatment of KD, and complications associated with KD, which may be a key direction for future KD research.

Looking at the results of the data analysis throughout the study, it is easy to see that there are indeed major differences between the WOS and Scopus databases. Previous studies also confirm our findings that Scopus provides 20% more coverage of citation information than Web of Science and that Scopus has a broader range of journals (55). When performing bibliometric analysis, searching only one of the databases may result in missing important data, and joint searching is recommended to broaden the scope of the search.

Although this study is the first bibliometric analysis of studies with KD as a topic in the last 5 years. And the research hotspots in the field were explored, which is informative. However, there are some limitations, such as the inclusion of only English articles in the analysis, which may have overlooked important research information in other languages. In this study, factors such as country, institution, journal, author, and keywords were analyzed at a macro level. However, the influence

of relevant medical policies, support funds, and government agencies on KD research needs further investigation.

Conclusion

This bibliometric analysis summarizes for the first time the research progress in KD (2017–2021), providing a qualitative and quantitative assessment of the bibliometric information on KD research. In this field, researchers mainly from Japan and USA are dominant, followed by China. It is recommended to pay close attention to the latest hotspots, such as "COVID-19" and "multisystem inflammatory syndrome." These results provide a more intuitive and convenient way for researchers to obtain the latest information on KD research.

Data availability statement

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

Author contributions

WT was responsible for most of the work including literature screening, data extraction, statistical analysis, paper writing, manuscript submission, and revision. LJ and YW were responsible for literature screening, image creation, and other necessary support. WL provided project proposal design, guidance, and financial support for this work. All authors contributed to the article and approved the submitted version.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1075659/full#supplementary-material

SUPPLEMENTARY TABLE S1
The most frequent keywords and their frequency.

SUPPLEMENTARY TABLE S2
Author keyword cluster of KD research.

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Hemolytic anemia following intravenous immunoglobulins in children with PIMS-TS: Two case reports

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This is the first case report on two children presenting with immediate and severe hemolytic anemia following the administration of high-dose intravenous immunoglobulins (IVIGs) in the context of pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS). Hemolytic anemia was described as a significant decrease in hemoglobin and an increase in lactate dehydrogenase after the second administration of high-dose IVIGs was performed. Both patients were found to have AB blood group. One of our patients showed massive pallor, weakness, and inability to walk in association with hemolysis. However, in both cases, the anemia was self-limiting and transfusion of red blood cells was not required: both patients recovered without persistent impact. Nonetheless, we aim to draw attention to this widely unknown adverse effect of IVIG, especially in the context of PIMS-TS. We suggest determining the patient's blood group prior to high-dose IVIG infusion and replacing the second IVIG through high-dose steroids or anticytokine therapy. Using IVIGs containing lower titers of specifically anti-A or anti-B antibodies to avoid isoagglutinin-caused hemolytic anemia is desirable; however, the information is not routinely available.

KEYWORDS

SARS-CoV-2, PIMS-TS, MIS-C, hemolytic anemia, blood group, IVIG (intravenous immunoglobulin) administration

Introduction

Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) is a severe hyperinflammatory condition with multiorgan involvement, also known as multisystem inflammatory syndrome associated with coronavirus disease 2019 (MIS-C), developing about 3–6 weeks after an acute or subclinical SARS-CoV-2 infection. Data on the incidence of PIMS-TS are rare, but PIMS-TS is reported to occur in approximately 4.5 per 10,000 children infected with SARS-CoV-2 in Great Britain (1).

PIMS-TS presents with symptoms resembling Kawasaki disease (KD) (2). PIMS-TS is not contagious and represents a delayed hyperinflammation process with cytokine storm (3). Levels of C-reactive protein (CRP), ferritin, and D-dimers, for example, have been

reported to be higher in PIMS-TS than those in KD and represent the overactivation of the immune system (3, 4). The diagnosis of PIMS-TS can be confirmed if the following criteria are met: (1) fever > 48 h, (2) elevated inflammatory values such as C-reactive protein, (3) at least two-organ involvement (conjunctivitis, exanthema, gastrointestinal symptoms like diarrhea, abdominal pain, and others), (4) positive SARS-CoV-2 polymerase chain reaction (PCR) or point of care (PoC) antigen tests, positive SARS-CoV-2 antibodies (IgM or IgG), or recent contact with an infected person with SARS-CoV-2, and (5) exclusion of other causes of infection (5). Hyperinflammatory conditions like KD and PIMS-TS are often treated with immunomodulating therapeutic measures like IVIGs. Side effects are mainly headache and hyperviscosity. Hemolytic anemia has been reported in association with high-dose treatment with IVIG; however, high concentrations of isoagglutinins were identified as causal factors in patients with Kawasaki disease and non-O blood groups (6). Others reported autoimmune responses to blood group antigens (7). This is the first case report on two children presenting with hemolytic anemia following the administration of IVIGs in the context of PIMS-TS.

Case presentation

Patient 1, a 2-year-old girl, presented to our emergency department with a high fever of up to 40°C for 5 days, abdominal pain, irritability, and glazed lips. Immediately before admission, she also had a sore throat, mildly enlarged submandibular lymph nodes, and a red, target-shaped efflorescence on the right cheek. Her height, weight, and body mass index were at the 89th, 86th, and 69th percentiles, respectively. The child had an elevated pulse rate during fever episodes and age-appropriate blood pressure. Clinical examination revealed regular auscultation of the heart and the lung and no signs of hepatosplenomegaly, which was confirmed by abdominal ultrasound. The initial CRP was elevated, with levels up to 140 mg/ml, and D-dimers surpassed the reference range >35 mg/L (**Table 1**). Furthermore, lymphopenia (19%, normal range 22–59) and neutrophilia (75%, normal range 25–74) were noticed. A known SARS-CoV-2 contact was reported 3 weeks before; however, only minor symptoms were observed afterward. The further workup with echocardiography showed a conspicuous but minimal dilatation of the left coronary artery (3 mm). The ejection fraction was normal. The heart ultrasound normalized 9 months after admission. The patient did not have any pre-existing conditions. Antipyretics but no other medications were administered prior to hospital admission. The psychosocial circumstances were unremarkable, with normal development.

Patient 2, an 8-year-old girl, was admitted to our emergency department with a fever of up to 39°C for 3 days, dry cough, chest pain, headache, and neck pain with cervical lymphadenopathy and suspected meningism as well as tonsil swelling. The strep A test was negative in the emergency room.

TABLE 1 Characteristics.

	Patient 1: 2 years, 4m-old female			Patient 2: 8 years, 4m-old female		
Onset coronavirus infection	03/2021			08/2021		
Onset PIMS-TS	~3 weeks after infection			~3 weeks after infection		
Clinical appearance of PIMS-TS	Recurrent fever episodes > 40°C without response to antipyresis for 5 days, sore throat, submandibular lymph nodes, abdominal colics; irritability; glazed lips, target-shaped efflorescence on right cheek			Recurrent fever episodes up to 39°C for 3 days, dry cough, headache, neck pain, sore throat, cervical lymphadenopathy, swollen tonsils, suspected meningism, chest pain		
Blood group	AB, RhD-positive			AB, RhD-positive		
Irregular isoagglutinins	Anti-A1/anti-A2			Anti-A1		
DAT	IgG+			IgG+		
	Laboratory at admission	Laboratory 5 days after 2nd IVIG	Laboratory 30 days after 2nd IVIG	Laboratory at admission	Laboratory 3 days after 2nd IVIG	Laboratory 20 days after 2nd IVIG
SARS-CoV-2 IgG/IgA ELISA	Positive/positive	—	—	Positive/positive	—	—
Hemoglobin (11.2–14.6 g/dl)	10.4[a]	5.7[a]	12.6	11.5	6.8[a]	8.9[a]
LDH (120–300 U/L)	247	1101[a]	397[a]	239	486[a]	307[a]
Haptoglobin (0.3–2 g/L)	—	<0.1[a]	—	—	<0.1[a]	<0.1[a]
CRP (<5 mg/ml)	140[a]	4.9	<1	243[a]	55.9[a]	1.7
Ferritin (7–84 µg/L)	470.3[a]	—	43.8	257.3[a]	730.7[a]	221.7[a]
D-dimers (<0.50 mg/L)	>35[a]	0.51[a]	>35[a]	1.12[a]	2.99[a]	—
Factor VIII (70%–150%)	204[a]	—	—	275[a]	—	—
vW antigen (56%–162%)	226.6[a]	—	—	291.9[a]	—	—
Troponin T (<14 ng/L)	<3	<3	—	22[a]	7	—
NT-pro-BNP[b] ng/L—*no references*	139	399	—	15,960	1,656	—

PIMS-TS, pediatric inflammatory multisystem syndrome temporally associated with SarsCov2; DAT, direct antiglobulin test; IVIG, intravenous immunoglobulin; CRP, C-reactive protein; LDH, lactate dehydrogenase.
[a]Out of reference range.
[b]Age-dependent reference range for NT-pro-BNP.

Her height, weight, and body mass index were at the 91th, 80th, and 63th percentiles, respectively. An elevated pulse rate during fever episodes and subnormal blood pressure were noticed. The clinical examination revealed regular lung and heart auscultation and a slightly distended abdomen. The abdominal ultrasound did not reveal hepatosplenomegaly. In addition to discrete palmar erythema, no skin lesions were noticed. The patient suffered from a reduced general condition. CRP levels were elevated up to 243 mg/ml. Blood cell count showed up to 84% neutrophilia and lymphopenia with the lowest value of 15%. Cerebrospinal fluid results were unremarkable. Vitamin D3 level was low (14 nmol/L). A known SARS-CoV-2 contact was reported approximately 3 weeks before. Echocardiography was initially normal, with the ejection fraction at the lower limit of normal. However, pro-BNP was elevated (15,960 ng/L). Soon, a mild dilatation of the coronary arteries (3–4 mm) and a minor pericardial effusion were observed. The ejection fraction was normal. The mean arterial pressure (MAP) was decreased by 46 mmHg. The heart ultrasound normalized already 7 days after admission. Patient 2 did not have any pre-existing conditions or regular medications. She lived with a single mother and, as far as known, was of unremarkable psychosocial condition and normal development.

We diagnosed PIMS-TS according to patients' symptoms and laboratory parameters, such as elevated CRP, neutrophilia and lymphopenia, markers of acute-phase reactions (factor VIII, vW antigen), D-dimers, and especially positive SARS-CoV-2 IgG antibodies, presumably due to reported SARS-CoV-2 some weeks before (**Table 1**). Both patients showed two affected organ systems. Furthermore, we excluded other causes of infections like potential bacterial and virus infections, e.g., cytomegalovirus, Epstein–Barr virus, and adenovirus.

Initially, both patients received intravenous broad-spectrum antibiotic therapy due to a high fever of unknown origin. Additionally, patient 2 received antiviral medications due to suspected encephalitis at admission. Following confirmation of PIMS-TS, anti-inflammatory treatment with prednisolone (2 mg/kg b.w./day) was administered, in addition to acetylsalicylic acid (5 mg/kg b.w./day), due to the mildly enlarged coronary arteries and prophylactic subcutaneous low-molecular-weight heparins (1 mg/kg b.w./day). The initial dose of IVIGs (2 g/kg b.w./day) was administered within the first 4 days after admission (**Figure 1**).

Anti-inflammatory treatment with prednisolone was given for almost 3 weeks to both patients. Cardioprotective acetylsalicylic acid was administered for 10 months to patient 1 and for 5 weeks to patient 2. Subcutaneous low-molecular-weight heparins were administered for 3 weeks to patient 1 and for 5 weeks to patient 2.

After a short, afebrile period, both patients suffered a relapse with high fever and reduced general condition. Therefore, both received a second administration of IVIGs within 10 days after the onset of symptoms (**Figure 1**). Patient 1 received the second IVIGs approximately 40 h after the first administration, whereas patient 2 received the second IVIG administration 4 days after the first dose (**Figure 1**).

Subsequently, patient 1 presented with symptomatic hemolytic anemia with pallor, massive weakness, inability to walk, and decreased hemoglobin (Hb) (from 10.4 to 5.8 g/dl), increased lactate dehydrogenase (LDH) (247–1,101 U/L), and nondetectable haptoglobin (**Table 1**, **Figure 1**). Patient 2 developed a complicated course, requiring intermediate care (IMC) for 6 days due to blood pressure instability. Being immobilized on IMC, the symptoms of hemolytic anemia were clinically not as evident in patient 2 as in patient 1. Beyond that, patient 2 suffered from edema, requiring the intake of diuretics. Furthermore, patient 2 received an interleukin-1 receptor antagonist (anakinra; 100 mg subcutaneously/three times/week) for 4 weeks due to a further recurrent fever episode after the second administration of IVIGs. In both patients, the anemia was self-limiting and did not require transfusion of red blood cells (RBCs). At the last follow-up (10 months after the onset for patient 1, 5 months after the onset for patient 2), both patients were symptom-free and did not take any medication.

The blood group of both children was determined as AB RhD-positive. However, irregular isoagglutinins were detectable in the plasma of both children (anti-A1 and anti-A2 in patient 1 and anti-A1 in patient 2). In addition, the direct antiglobulin test (DAT) was positive for IgG in both patients, leading to the diagnosis of IVIG-associated hemolysis. No other irregular allo- or autoantibodies to RBCs were detected in the plasma or eluate of either patient. The anti-A isoagglutinins were most likely transmitted with the IVIG batch, causing severe hemolysis. Subsequently, we compared the anti-A and anti-B titers of the administered preparation with another commercially available preparation. The anti-A1 IgG titers of 1:128 were significantly higher in the preparation that both patients received than the anti-A1 titers (1 : 16) in an IVIG preparation from another manufacturer.

The outcome of both children during the follow-up of 10 and 5 months, respectively, was excellent. Patient 1 could be discharged home after almost 2 weeks of inpatient treatment, and patient 2 could be discharged home after almost 3 weeks. Thus, the hemolysis due to the treatment with IVIGs did not prolong the hospital stay. The patients and parents were fully informed about hemolysis as a potential side effect of IVIGs.

Both children were seen for regular follow-ups including blood tests and echocardiography in our outpatient clinic after discharge. There were no long-term consequences regarding anemia, inflammatory values, physical conditions, or pathologies in the heart ultrasound. However, patient 2 suffered from fatigue for another 6 weeks after discharge.

Discussion

IVIG-associated hemolysis appears to be associated with high-dose IVIG >1 g/kg, non-O blood group, and severe inflammatory disease (6, 7). We emphasize that blood group AB, as in both our patients, shows the highest risk of developing hemolytic anemia and that blood group O is almost unaffected in IVIG-associated hemolysis (6). The prevalence of IVIG-associated hemolysis emerged with newer IVIG preparations after 2007, and the prevalence of IVIG-associated hemolysis, especially in KD, shows

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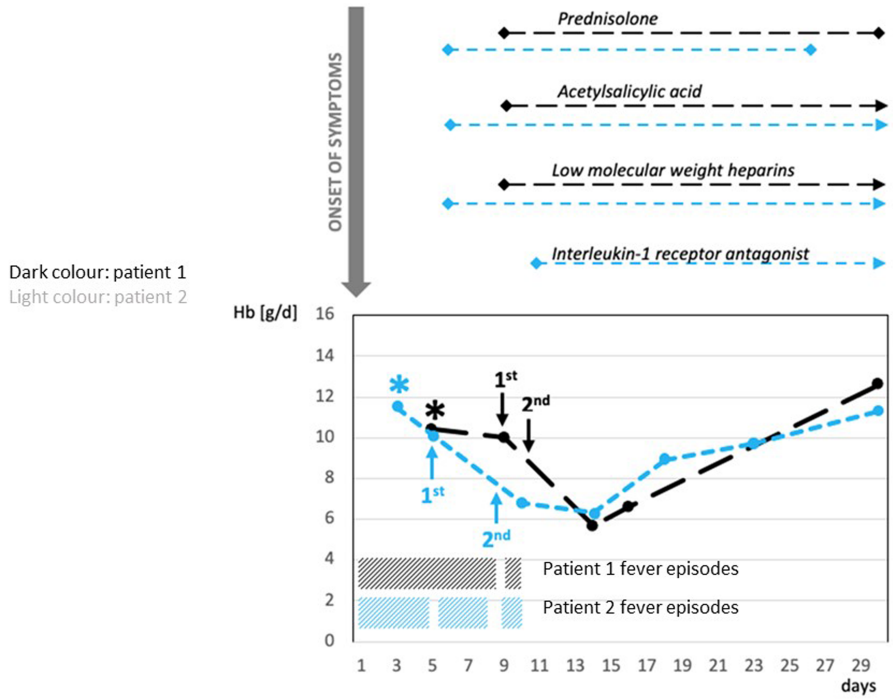

FIGURE 1
Correlation between administration of IVIG and severe hemolytic anemia: time course of Hb, treatment of PIMS-TS, and recurrent fever episodes. *admission to hospital; *Prednisolone* (2 mg/kg/day): Patient 1 and Patient 2 for 3 weeks; *Acetylsalicylic acid* (5 mg/kg b.w./day): Patient 1 for 10 months, Patient 2 for 5 weeks; *Low-molecular-weight heparins* (1 mg/kg b.w./d): Patient 1 for 3 weeks, Patient 2 for 5 weeks; *Interleukin-1 receptor antagonist* (anakinra—100 mg subcutaneously/3 times/week): Patient 2 for 4 weeks. Patient 1: first administration of IVIG 2 g/kg—9 days after onset of PIMS-TS; second administration of IVIG 2 g/kg about 40 h after the first administration. Patient 2: first administration of IVIG 2 g/kg 5 days after onset of PIMS-TS; second administration of IVIG 2 g/kg 4 days after the first administration. PIMS-TS, pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV2; IVIG, intravenous immunoglobulin; Hb, hemoglobin.

a variation between 0.36% and 16% (6). Pathophysiologically, IVIG products presumably cause opsonization of erythrocytes, undergoing FcgRIIa-dependent phagocytosis by activated macrophages, leading to extravascular hemolytic anemia. This opsonization *via* isoagglutinins anti-A and anti-B subclass IgG2 in IVIG products is enhanced in the presence of proinflammatory cytokines (6). Thus, inflammatory disorders like PIMS-TS and KD enhance the potential risk of isoagglutinin-mediated hemolysis. Furthermore, high anti-A-IgG titers in IVIGs can provoke hemolysis (8). Although patient 2 showed a slight decrease in hemoglobin before the start of IVIGs, assumingly due to the severe inflammation, hemolysis played the major role in the decrease of hemoglobin.

In KD, in addition to alloimmune hemolysis by passively transferred isoagglutinins (anti-A and anti-B), a potentially autoimmune-mediated mechanism by anti-M or anti-C/-c antibodies has been postulated in individual case reports (7, 9). However, in these publications, hemolysis lasted much longer than in our two cases, and blood transfusion was needed in one case, indicating a more severe clinical presentation. Furthermore, in our two children, additional irregular allo- or autoantibodies to blood groups were excluded.

Referring to the data from the Italian observational multicenter retrospective study comparing KD and KD-like multi-inflammatory syndrome diagnosis in association with SARS-CoV-2 (KawaCOVID), our patients fitted into the KawaCOVID group based on the presence of persistent fever (>48 h), lymphopenia, and evidence of single- or multiorgan dysfunction (2). Neither child showed major skin symptoms; the younger child (case 1) had only mild cheilitis and a single target-shaped efflorescence on the right cheek. The older child had very discrete palmary erythema. Patient 2 showed hypotension, which was rarely seen in KD, and required intermediate care.

The superiority of an immediate prednisolone therapy for PIMS-TS, as performed in the reported children, in addition to the initial IVIG application, has been demonstrated (10, 11). At the time our two patients were admitted to the hospital, the treatment guidelines for Kawasaki-like PIMS-TS recommended the use of high-dose methylprednisolone, an anticytokine therapy (e.g., anakinra, tocilizumab, or infliximab), or a second IVIG dose in the case of an insufficient response to the first IVIG application (12, 13). Based on the observed significant adverse effects of the second IVIG application, published data, and our own experience, we would currently recommend the use of high-dose steroid therapy or anakinra instead of the second IVIG dosage (14, 15). If the second dose of IVIGs is still indicated, we advocate intervals of $\geq$ 48 h between the first and second doses to reduce the cumulative dose per time and to have the chance of early detection of potential hemolysis after the first dose.

In doing so, we refer especially to possible high-risk patients with AB blood group. We propose ABO blood group typing *before* the administration of high-dose IVIGs to first carefully evaluate the indication of IVIG administration in AB blood group and second in the case of subsequent blood transfusion because of potential isoagglutinins counteracting blood group analysis. The current requirements for the maximum titer (1:64) of anti-A and anti-B in IVIG preparations should also be considered by the manufacturers and authorities (6). Furthermore, we suggest monitoring patients with severe inflammatory syndromes carefully for hemolytic anemia 3–5 days after IVIG administration regarding Hb, LDH, and haptoglobin.

If hemolysis occurs after high-dose IVIGs and autoantibodies against RBCs are detected in addition to a positive DAT, autoimmune-mediated hemolysis should also be considered a rare differential diagnosis (7, 9), especially if it is long-lasting and severe.

We conclude that IVIG-associated hemolysis can appear more probably after the second administration of IVIGs within less than 48 h (dose per time) due to a high cumulative effect. Additionally, we assume that IVIG-associated hemolysis could be caused by a single high-dose IVIG if there are excessive isoagglutinins in the batch (>1:64), especially anti-A.

Our case reports should be considered in light of several limitations. First, we emphasize that our observation of hemolytic anemia following IVIG in the context of PIMS-TS has not been empirically verified since our report is limited regarding the small number of cases. Second, PIMS-TS in our two patients appeared in times of the emerging predominance of SARS-CoV-2 B1.1.7. The appearance of B1.1.7 in Germany correlated with rising numbers of PIMS-TS (16). Currently, in winter 2022/2023, omicron subvariants prevail and the incidence of PIMS-TS has been decreasing in Germany since the beginning of 2022 (16). Yet, it remains unpredictable if PIMS-TS will come up again due to new virus variants of SARS-CoV-2.

Data availability statement

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

Ethics statement

Charité Ethics Committee confirmed that no approval is needed for case reports if written informed consent to publish these data of parents/legal guardian/next of kin is provided. Written informed consent to publish clinical data of the patients was provided by the participants' legal gurdian/next of kin.

Author contributions

SL: edited and approved the manuscript and will be accountable for manuscript integrity. ES, BM, and SL: project conception and manuscript drafting, editing, and approval. HvB and TK: manuscript editing and approval. All authors contributed to the article and approved the submitted version.

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Conflict of interest

HvB was employed by Labor Berlin GmbH.

The remaining 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.

Publisher's note

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Platelet role in the prediction of MIS-C severity

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Introduction: Multisystem inflammatory syndrome in children (MIS-C) has been reported as one of the cytokine storm syndromes associated with COVID-19. Despite the several proposed diagnostic criteria, MIS-C remains a diagnostic and clinical challenge. Recent studies have demonstrated that platelets (PLTs) play a crucial role in COVID-19 infection and its prognosis. This study aimed to investigate the clinical importance of PLT count and PLT indices in predicting MIS-C severity in children.

Patients and methods: We conducted a retrospective single-center study at our university hospital. A total of 43 patients diagnosed with MIS-C during a 2-year period (from October 2020 to October 2022) were included in the study. MIS-C severity was evaluated according to the composite severity score.

Results: Half of the patients were treated in the pediatric intensive care unit. No single clinical sign was associated with a severe condition, except for shock ($p = 0.041$). All the routine biomarkers, such as complete blood count (CBC) and C-reactive protein (CRP), used for MIS-C diagnosis were significant in predicting MIS-C severity. Single PLT parameters, such as mean PLT volume, plateletcrit, or PLT distribution width, did not differ between the severity groups. However, we found that a combination of PLT count and the previously mentioned PLT indices had the potential to predict MIS-C severity.

Conclusions: Our study emphasizes the importance of PLT in MIS-C pathogenesis and severity. It revealed that together with routine biomarkers (e.g., CBC and CRP), it could highly improve the prediction of MIS-C severity.

KEYWORDS

multisystem inflammatory syndrome in children, COVID-19, biomarker, children, platelets

1. Introduction

During the COVID-19 pandemic, severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection in children was and is related to a less severe disease compared with that in adults. However, some children also develop COVID-19-related sequelae. Multisystem inflammatory syndrome in children (MIS-C) has been reported as one of the cytokine

Abbreviations

ALT, alanine transaminase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; AUC, area under the ROC curve; CBC, complete blood count; CI, confidence intervals; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyl transferase; GSATool, Gaslini severity assessment tool; Hgb, hemoglobin; INR, international normalized ratio; IQR, interquartile range; KD, Kawasaki disease; LDH, lactate dehydrogenase; Lymph, lymphocyte; LOS, length of stay; MAS, macrophage activation syndrome; MIS-C, multisystem inflammatory syndrome in children; Mono, monocyte; MPV, mean platelet volume; Neu, neutrophil; OR, odds ratio; PCT**, procalcitonin; PCT, plateletcrit; PCR, polymerase chain reaction; PDW, platelet distribution width; PICU, pediatric intensive care unit; PLT, platelet; pro-BNP, NT-proB-type natriuretic peptide; PT, prothrombin time; PVI, platelet volume indices; ROC, receiver operating characteristic; SD, standard deviation; WBCs, white blood cells; WHO, World Health Organization.

storm syndromes associated with COVID-19 and also in most of the patients who partially or fully meet the criteria of Kawasaki disease (KD) (1). Despite the several proposed diagnostic criteria (2–5), MIS-C remains a diagnostic and clinical challenge. This is evident in the variety of clinical MIS-C presentations, which can mimic different conditions from macrophage activation syndrome (MAS)-like features to less severe disease phenotypes, contributing to diagnostic complexity (6). The main symptoms of MIS-C, such as fever, rash, and signs of systemic inflammation resulting in multisystem organ dysfunction, can be found in many other infectious or inflammatory diseases in children (7). Nevertheless, a clear relationship between MIS-C outbreaks and previous SARS-CoV-2 infections defines it as a postinfectious condition (8–12). In the last few years, MIS-C has been scored according to its severity. Some patients demonstrate mild systemic symptoms and prolonged fever, while others present with signs of cardiac injury and shock, leading to admission to a pediatric intensive care unit (PICU) (6, 11). To date, efforts are ongoing to understand the pathogenesis of this condition and analyze any biomarkers that could predict the severity and outcomes of the disease.

One of the pathogenesis arms in severe SARS-CoV-2 infection and MIS-C is the activation of coagulation, resulting in a prothrombotic state in severe patients (13–15). The role of activated PLTs in the stimulation of immune cells and their interaction with different pathogens has been well described in several studies (16–18). For example, PLTs are involved in the formation of neutrophil (Neu) extracellular traps and in phagocytosis (19, 20). PLT counts and PLT indices, such as mean PLT volume (MPV) and PLT distribution width (PDW), which are easily obtainable from routine blood samples, are widely discussed as biomarkers and prognostic factors in severe bacterial infections, such as sepsis (21, 22). Moreover, they are promising markers for distinguishing viral infections, such as flu and COVID-19 (23). In addition, recent studies have demonstrated that PLTs play a crucial role in COVID-19 infection and its prognosis (24, 25). Only a few studies discuss PLT count as a risk factor for MIS-C and its severity (26–29). Thus, we aimed to investigate the clinical importance of PLT count and PLT indices in predicting MIS-C severity in children who presented to our university hospital and were diagnosed with MIS-C.

2. Materials and methods

2.1. Study design and study population

This retrospective single-center study was conducted at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos. Data of all patients who met the World Health Organization (WHO) case definition of MIS-C (4) referred and treated from 1 October 2020 to 1 October 2022 were analyzed.

2.2. Data collection

Demographic data (age, gender), living location, past medical history, and data regarding previous COVID-19 infection (infection, contact, and duration from the contact) were collected from an electronic data system. The day of contact with the virus was defined according to the positive epidemiological anamnesis of confirmed COVID-19 illness in close relatives of the same household or school or other close contacts. Moreover, we included admission to the PICU, total length of stay (LOS) in the ward, and LOS in the PICU in the data analysis. In addition, MIS-C severity was evaluated according to the composite severity score proposed by Brisca et al.—Gaslini severity assessment tool (GSATool) (30). The groups were defined according to clinical presentation, signs of cardiac dysfunction on echocardiography, increased cardiac enzyme levels, and signs of laboratory features of MAS (30). Instead of using four severity classes as described in the original study, because of the small sample size of the study, we divided the patients into two groups: Group 1 (less severe patients corresponding to classes I and II according to the GSATool) and Group 2 (severe patients representing classes III and IV of GSATool) (30).

2.3. Laboratory tests

Considering the presentation signs of MIS-C, routine markers as follows were obtained: complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin (PCT**), lactate dehydrogenase (LDH), albumin, creatine, urea, alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cardiac dysfunction markers [troponin I, NT-proB-type natriuretic peptide (pro-BNP)], evidence of coagulopathy [prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), fibrinogen (FB), and elevated D-dimer (DD) levels], and signs of possible macrophage activation [ferritin (FR)]. The results of all the mentioned biomarkers were included in the data analysis. From the initial CBC, white blood cell (WBC), Neu, lymphocyte (Lymph), PLT count, hemoglobin (Hgb), MPV, plateletcrit (PCT), and PDW data were collected.

2.4. Statistical analysis

Statistical analysis was performed using Microsoft Excel and IBM SPSS Statistics version 29.0 software (SPSS Inc., Chicago, IL, United States) for Windows. We used the Shapiro–Wilk test to determine whether the data were normally distributed. Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR). Qualitative data were presented as counts and percentages (%). As mentioned previously, the whole cohort was subdivided into two groups according to the MIS-C severity class of GSATool: Group 1 (GSATool class I and class II) and Group 2 (GSATool

class III and class IV). Both groups were compared by an independent samples *t*-test if the data were normally distributed and by the Wilcoxon signed rank test and Mann–Whitney *U* test for non-parametric data. The diagnostic accuracy of MIS-C biomarkers was compared using receiver operating characteristic (ROC) curves. Youden's index was used to determine the cutoff values. A p-value < 0.05 was considered significant.

2.5. Ethical consent

Permission to conduct the study was obtained from the Kaunas Regional Biomedical Research Ethics Committee (BE-2–27). This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

3. Results

3.1. General characteristics

In total, 43 children with a median age of 8 (5.2–10.9) years were included in the study. Only 13 pediatric patients matched GSATool classes III and IV and were assigned to Group 2. Older children had a more severe MIS-C course ($p = 0.046$). In total, 67.4% were boys, and a higher percentage of males was found in both severity groups. Half of the children were from the city (Table 1). More children with a lower MIS-C score were present in the Kaunas City area, but this was not significant ($p = 0.587$). Only seven children indicated a previous COVID-19 infection; however, data were lacking in the majority of the cases. Twenty children had previous contact with COVID-19-infected persons (relative, at school, etc.), and this did not differ significantly between the two groups. The median duration from contact to the first symptoms was 27.5 (25–30) days (Table 1), with no difference between cohorts ($p = 0.253$). Approximately half (48.8%) of the referred children were admitted to the PICU, with a median LOS of 2 (1–4) days in the PICU. More children were admitted in Group 2, but the difference was not significant ($p = 0.078$). Altogether, the median LOS was 10 (7–12) days. A significantly longer LOS was observed in more severe cases [9 (7.8–10.8) vs. 15 (10.9–19.6), respectively, $p = 0.004$] with no difference in the PICU LOS ($p = 0.547$) (Table 2).

3.2. Clinical features

On referral, most frequently, the children complained of gastrointestinal symptoms, rash, and conjunctivitis (Table 2). Twelve children had clinical features of pneumonia, and four presented with shock (Table 2). None of the symptoms or clinical features differed significantly between the severity groups, except for shock ($p = 0.041$). However, more severe MIS-C cases

TABLE 1 General characteristics of total MIS-C patients and according to the severity groups.

Number of patients included (*n*)		Total (*n* = 43)	Group 1 *n* = 30	Group 2 *n* = 13	*p*-value
Median age (year) (IQR)		**8 (5.2–10.9)**	**7 (6.9–8.8)**	**10 (7.6–12.6)**	**0.046**
Age groups (year) (%)	<5	10 (23)	9 (30)	1 (7.7)	0.422
	5–10	18 (41.9)	12 (40)	6 (46.2)	
	10–15	13 (30.2)	8 (26.7)	5 (38.5)	
	>15	2 (4.7)	1 (3.3)	1 (7.7)	
Gender	Gender (male) (%)	29 (67.4)	21 (70)	8 (61.5)	0.587
Location	Region (%)	20 (46.5)	12 (40)	8 (61.5)	0.193
	City (%)	23 (53.5)	18 (60)	5 (38.5)	
SARS-CoV-2 infection	Positive PCR (%)	7 (16.3)	4 (13.3)	3 (23.1)	0.091
	Not detected (%)	5 (11.6)	5 (16.7)	0 (0)	
	No data (%)	31 (72)	21 (70)	10 (76.9)	
COVID contact	Positive (%)	20 (46.5)	16 (53.3)	4 (30.8)	0.203
	Negative (%)	21 (48.8)	13 (43.3)	8 (61.5)	
	No data (%)	2 (4.7)	1 (3.3)	1 (7.69)	
Median duration from contact to first MIS-C symptoms (day) (IQR)		27.5 (25–30)	5.63 (3.29–7.97)	4.46 (3.12–5.80)	0.253
Severity score (*n*, %)	0	15 (34.9)			
	I	6 (14.0)			
	II	9 (20.9)			
	III	10 (23.3)			
	IV	3 (7.0)			
Admitted to PICU (*n*) (%)		21 (48.8)	12 (40)	9 (69.2)	0.078
Median LOS (day) (IQR)		**10 (7–12)**	**9.30 (7.81–10.79)**	**15.23 (10.87–19.60)**	**0.004**
Median LOS in PICU (day) (IQR)		2 (1–4)	2.36 (1.27–3.46)	3.38 (1.65–5.10)	0.547

n, number; IQR, interquartile range; SD, standard deviation; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus; COVID, coronavirus disease; MIS-C, multisystem inflammatory syndrome in children; PICU, pediatric intensive care unit; LOS, length of stay.
p-value < 0.05 was considered statistically significant. Statistically significant results are provided in bold.

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TABLE 2 Symptoms and clinical features of total MIS-C patients according to the severity groups.

Symptoms and clinical features	Total, *n*	Severity group		*p*-value
	43	Group 1 (*n* = 30)	Group 2 (*n* = 13)	
Vomit, *n* (%)	21 (49)	14 (47)	7 (54)	0.665
Abdominal pain, *n* (%)	24 (56)	15 (50)	9 (70)	0.244
Diarrhea, *n* (%)	16 (37)	10 (33)	6 (46)	0.424
Obstipation, *n* (%)	2 (5)	2 (7)	0	0.342
Mesadenitis, *n* (%)	13 (30)	7 (23)	6 (46)	0.135
Lymphadenopathy, *n* (%)	7 (16)	5 (17)	2 (15)	0.917
Conjunctivitis, *n* (%)	25 (58)	17 (57)	8 (62)	0.766
Rash, *n* (%)	31 (72)	22 (73)	9 (69)	0.783
Sole desquamation, *n* (%)	4 (9)	3 (10)	1 (8)	0.811
Raspberry lips, tongue, *n* (%)	19 (44)	13 (43)	6 (46)	0.864
Pneumonia, *n* (%)	12 (28)	7 (23)	5 (38)	0.311
Pleuritis, *n* (%)	5 (12)	3 (10)	2 (15)	0.698
Bronchitis, *n* (%)	8 (19)	3 (10)	5 (38)	0.058
Coronary injury, *n* (%)	4 (9)	1 (3)	3 (23)	0.154
Other symptoms (neurological, nephrological, and articular), *n* (%)	9 (21)	6 (20)	3 (23)	0.312
Shock, *n* (%)	**4 (9)**	**1 (3)**	**3 (23)**	**0.041**

n, number; IQR, interquartile range; MIS-C, multisystem inflammatory syndrome in children.
p-value < 0.05 was considered statistically significant. Statistically significant results are provided in bold.

presented with abdominal pain and vomiting, and more cases had features of mesadenitis and bronchitis (Table 2).

3.3. Laboratory test results

Blood was drawn from all the patients for CBC, CRP, and other analyses of MIS-C biomarkers. Neu count was significantly higher in more severe MIS-C cases (Group 2) compared with that in Group 1 patients [10.4 (7.3–13.5) vs. 6.8 (5.4–8.2), respectively, $p = 0.013$] (Table 3). Other cell counts, Hgb, and PLT markers did not differ (Table 3). A significant increase in CRP levels was seen in more severe MIS-C cases ($p = 0.027$), along with other markers such as DD ($p = 0.027$), pro-BNP ($p = 0.013$), FR ($p = 0.002$), LDH ($p = 0.032$), and urea ($p = 0.049$) (Table 3).

3.4. Prediction of MIS-C severity

We analyzed different marker combinations for predicting MIS-C severity. First, routine blood biomarkers were investigated following the MIS-C guidelines. All combinations were significant in predicting a severe course of MIS-C (Table 4). We observed that the combination of CRP with Neu with a cutoff value of 0.834 and area under the ROC curve (AUC) of 0.774 had a specificity of 92.3%; however, the sensitivity was 60% (Table 4). When biomarkers were added stepwise, sensitivity and specificity increased, with the best results obtained when all the biomarkers according to the MIS-C diagnostic guidelines were included (Table 4).

3.5. PLTs and PLT parameters

We analyzed the potential of PLT, MPV, PCT, and PDW in predicting MIS-C severity. With a cutoff value of 0.836 for the combination of these markers (without any additional CBC, CRP, and other biomarkers), the sensitivity and specificity for predicting MIS-C were 80.8% and 91.7%, respectively (AUC of 0.924; 95% CI of 0.940–1.009; $p < 0.001$) (Table 5). The inclusion of those markers along with the routine blood biomarkers enhanced their ability to predict MIS-C severity, increasing both sensitivity and specificity (Table 5).

4. Discussion

MIS-C is a cytokine storm–caused condition and requires immediate and aggressive treatment in severe cases. We evaluated a total of 43 MIS-C cases admitted and treated in our university hospital. All of the patients were previously healthy with no comorbidities. Children presented with a variety of symptoms, and half of them required treatment in PICU. Although a single PLT biomarker did not differ between the severity groups, we found that the combination of PLT count and PLT indices had the potential to predict MIS-C severity.

The risk of MIS-C has been influenced by the spread of different variants of SARS-CoV-2 virus. Various studies have identified that the incidence of MIS-C was markedly lower during the Omicron wave, compared with earlier variants of the virus (31–33). According to our study, nearly half (48%) of the MIS-C cases emerged following the initial wave of COVID-19 in Lithuania. Unfortunately, most of our patients did not undergo polymerase chain reaction (PCR) for SARS-CoV-2 before developing MIS-C. Thus, we relied on epidemiological anamnesis of confirmed COVID-19 illness in the close contacts. The median time to the MIS-C onset was around 4 weeks (27.5 days), which corresponds to the MIS-C outbreaks observed in the majority of previous studies (8, 10, 11, 26, 29, 34–36).

MIS-C is characterized by fever, overwhelming systemic inflammation, hypotension, and cardiac dysfunction. Recent studies show that the severity of this condition can range from mild cases with lower inflammation markers to the most severe cases presenting with shock and MAS-like features (6, 37, 38). Our analyzed cohort of children also covered this wide spectrum of presentations and severities of MIS-C. The mean age of 8 years and male gender predominance (67.4%) found in our study were in line with the data from most MIS-C studies (6, 10, 11, 13, 26, 29, 34–36, 39). Moreover, the leading clinical manifestations of MIS-C were rash, gastrointestinal symptoms, and conjunctivitis (Table 2). In general, all clinical signs were consistent with previously published MIS-C studies and case reports of predominant abdominal symptoms, rash, and cardiovascular involvement (6, 39–41). Various data show that approximately 30%–60% of patients present with symptoms of shock and the need for treatment in the PICU (10, 26, 29, 34). In our case, only four children had symptoms of shock. In

TABLE 3 Laboratory test results of total MIS-C patients on admission and according to the severity groups.

Laboratory tests	Total	Severity group		*p*-value
CBC	(*n* = 43)	Group 1 (median, IQR)	Group 2 (median, IQR)	
WBC, 10^9 cells/L	9.2 (6–12)	9.1 (7.5–10.8)	12.1 (8.6–15.5)	0.083
Neu (×10^9 cells/L)	**7 (4–11)**	**6.8 (5.4–8.2)**	**10.4 (7.3–13.5)**	**0.013**
Lymph (×10^9 cells/L)	1.4 (0.5–1.6)	1.6 (1.1–2.1)	1 (0.6–1.4)	0.160
Hgb (g/L)	120 (111–126)	118 (113.8–122.4)	122 (113.7–130.8)	0.321
PLT (×10^9 cells/L)	194 (102–303)	237 (174.3–299.2)	221 (129.2–313.2)	0.776
MPV (fl)	8.35 (7.2–9.4)	8.08 (7.61–8.56)	8.77 (8.25–9.28)	0.076
PCT (%)	0.17 (011–0.24)	0.18 (014–0.23)	0.21 (0.13–0.29)	0.538
PDW (%)	16.6 (16.3–17.0)	16.60 (16.42–16.77)	16.90 (16.57–17.23)	0.068
CRP (*n* = 43) (mg/L)	**145.8 (100–222)**	**135.1 (103.9–166.2)**	**199.9 (145.7–254.1)**	**0.027**
ESR (*n* = 41) (mm/h)	27 (14–41)	28.66 (18.75–38.56)	34.31 (20.82–47.80)	0.498
PCT** (*n* = 38) (ng/ml)	1.58 (0–7.75)	4.81 (1.11–8.52)	12.41 (2.22–22.59)	0.071
PT (*n* = 42) (%)	28.4 (24–34)	29.13 (22.94–35.32)	35.92 (26.27–45.58)	0.216
APTT (*n* = 42) (s)	38 (34–43)	36.42 (31.96–40.89)	39.57 (35.28–43.86)	0.377
INR (*n* = 39)	1.2 (1.1–1.3)	1.16 (1.05–1.26)	1.25 (1.17–1.33)	0.247
D-dimers (*n* = 41) (ng/ml)	**3.1 (2.0–4.5)**	**3.01 (2.43–4.37)**	**4.78 (3.42–6.14)**	**0.027**
Fibrinogen (*n* = 39) (g/L)	4.3 (2.6–5.4)	3.40 (2.43–4.37)	4.78 (3.42–6.14)	0.094
Troponin I (*n* = 39) (ng/ml)	**0.06 (0.03–0.20)**	**0.13 (0.01–0.24)**	**4.42 (1.24–13.43)**	**0.002**
Pro-BNP (*n* = 38) (	**82 (0–221)**	**85.84 (44.03–127.65)**	**475.03 (27.31–922.75)**	**0.013**
Ferritin (*n* = 39) (mcg/L)	**155 (78–280)**	**150.56 (101.39–199.72)**	**409.85 (182.45–637.25)**	**0.002**
LDH (*n* = 39) (U/L)	**236 (0–303)**	**174.73 (123.91–225.55)**	**276.77 (185.33–368.21)**	**0.032**
Albumin (*n* = 39) (g/L)	27 (0–31)	19.68 (12.96–26.40)	20.95 (12.05–29.84)	0.818
Creatine (*n* = 41) (μmol/L)	39 (31–47)	39.66 (32.43–46.98)	47.00 (33.49–60.51)	0.283
Urea (*n* = 40) (U/L)	**4.0 (2.8–4.8)**	**3.70 (3.21–4.20)**	**4.87 (3.38–6.36)**	**0.049**
ALT (*n* = 40) (IU/L)	26 (18–43)	35.18 (24.46–45.90)	32.25 (23.64–40.86)	0.730
AST (*n* = 40) (U/L)	36 (28–49)	40.68 (31.44–49.92)	41.83 (34.20–49.47)	0.875
GGT (*n* = 38) (IU/L)	18.5 (12.5–39)	31.65 (15.26–48.05)	39.58 (18.70–40.46)	0.867

CBC, complete blood count; WBC, white blood cell; Lymph, lymphocytes; Mono, monocytes; Hgb, hemoglobin; PLT, thrombocytes; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PCT**, procalcitonin; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; pro-BNP, NT-proB-type natriuretic peptide; LDH, lactate dehydrogenase; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; MIS-C, multisystem inflammatory syndrome in children.
p-value < 0.05 was considered statistically significant. Statistically significant results are provided in bold.

TABLE 4 Analysis of different laboratory parameters in MIS-C severity prediction.

Laboratory parameters	Cutoff value	Youden's index	AUC (95% CI)	Sensitivity %	Specificity %	*p*-value
CRP + Neu	0.834	0.523	0.774 (0.636–0.912)	60	92.3	<0.001
CRP + Neu + PCT**	0.768	0.494	0.728 (0.566–0.890)	57.7	91.7	0.006
CRP + Neu + PCT** + DD	0.623	0.47	0.800 (0.647–0.953)	80	67	<0.001
CRP + Neu + PCT** + DD + FB	0.552	0.614	0.860 (0.736–0.984)	86.4	75	<0.001
CRP + Neu + PCT** + DD + FB + pro-BNP	0.774	0.69	0.902 (0.801–1.002)	77.3	91.7	<0.001
CRP + Neu + PCT** + DD + FB + pro-BNP + FR	0.807	0.773	0.917 (0.823–1.012)	77.3	100	<0.001
CRP + Neu + PCT** + DD + FB + pro-BNP + FR + LDH	0.613	0.861	0.965 (0.909–1.022)	95.2	90.9	<0.001
CRP + Neu + PCT** + DD + FB + pro-BNP + FR + LDH + U	0.500	1	1.000 (1.000–1.000)	100	100	<0.001
CRP + Neu + Lymph + FR + pro-BNP + PCT**	0.836	0.75	0.924 (0.840–1.009)	75.0	100	<0.001

CRP, C-reactive protein; Neu, neutrophils; PCT**, procalcitonin; DD, D-dimer; FB, fibrinogen; pro-BNP, NT-proB-type natriuretic peptide; FR, ferritin; LDH, lactate dehydrogenase; U, urea; Lymph, lymphocyte; AUC, area under the ROC curve; CI, confidence interval.
p-value < 0.05 was considered statistically significant.

general, by analyzing our data, we retrospectively used a specific scoring method to identify the most severe patients and investigate the possible prognostic factors that could be used in early risk evaluation. We used the composite severity score (further GSATool) proposed by Brisca et al. (30). The researchers demonstrated that by using this multistep early risk evaluation (GSATool) and aggressive therapeutic approach, all 23 patients included in their study avoided admission to the PICU, invasive mechanical ventilation, extracorporeal circulatory and respiratory support, or administration of inotropic drugs. Most of our analyzed MIS-C patients fell into the less severe group (classes I and II according to the GSATool) ($n = 30$), and the majority of the cases did not differ in clinical symptoms or features at presentation, except for signs of shock ($p = 0.041$).

Admission to the PICU is one of the most evaluated outcomes for MIS-C severity. Systematic reviews conducted by Hoste et al. and Radia et al. have reported that approximately 68%–74% of MIS-C patients need treatment in the intensive care unit, and

TABLE 5 Analysis of platelet marker combinations with different laboratory parameters in MIS-C severity prediction.

Laboratory parameters	Cutoff value	Youden's index	AUC (95% CI)	Sensitivity %	Specificity %	*p*-value
PLT + MPV + PCT + PDW	0.716	0.725	0.901 (0.803–0.998)	80.8	91.7	<0.001
PLT + MPV + PCT + CRP	0.810	0.615	0.827 (0.760–0.984)	61.5	100	<0.001
PLT + MPV + PCT + PCT**	0.687	0.773	0.917 (0.821–1.014)	77.3	100	<0.001
PLT + MPV + PCT + PDW + pro-BNP	0.667	0.71	0.929 (0.845–1.012)	85.7	85.3	<0.001
PLT + MPV + PCT + PDW + FR	0.759	0.87	0.949 (0.877–1.020)	87.0	100	<0.001
PLT + MPV + PCT + PDW + LDH	0.642	0.83	0.906 (0.794–1.018)	91.3	91.7	<0.001
PLT + MPV + PCT + PDW + U	0.788	0.75	0.886 (0.779–0.994)	75.0	100	<0.001
PLT + MPV + PCT + PDW + FB	0.616	0.697	0.917 (0.826–1.007_	86.4	83.3	<0.001
PLT + MPV + PCT + PDW + LDH + FR	0.567	0.814	0.944 (0.855–1.032)	90.5	90.9	<0.001
PLT + MPV + PCT + PDW + LDH + pro-BNP	0.500	1	1.000 (1.000–1.000)	100	100	<0.001

PLT, platelet; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; CRP, C-reactive protein; PCT**, procalcitonin; pro-BNP, NT-proB-type natriuretic peptide; FR, ferritin; LDH, lactate dehydrogenase; U, urea; FB, fibrinogen; MIS-C, multisystem inflammatory syndrome in children; AUC, area under the ROC curve; CI, confidence interval.
p-value < 0.05 was considered statistically significant.

more than half of them (56.3%–77%) present with shock (13, 38). Another group of patients deteriorated during the disease course and were transferred to the PICU mainly because of the need for inotropic treatment (26, 38). In our study, nearly half of the children (48.8%) were admitted to the PICU. We observed that children were more frequently treated in the PICU if they scored higher according to the GSATool, although this was not statistically significant. Interestingly, 40% of Group 1 patients were admitted to the PICU despite the absence of the need for respiratory or cardiovascular support, which suggests that referral to the PICU may be influenced by the subjective decision of different physicians and the lack of specific severity (and/or outcome) prognostic factors in the early stage of the disease. Similar data were reported by Kaidar et al. in a retrospective multicenter study where one-third of the cases were treated in the PICU without the need for inotropic medications or vasopressors (26). The researchers hypothesized that admission to the PICU may not be the best outcome measure for MIS-C severity.

Nevertheless, the recovery from the severe condition is quite fast as the median duration in hospital in most of the studies does not exceed 10 days (11, 13, 27, 36, 42). We found similar results, with a short LOS in the PICU with a median of 2 days and an overall LOS in the hospital of a median of 10 days. The total LOS was mostly influenced by the severity group and admission to the PICU.

Regarding the variety of presentation signs of MIS-C, all patients underwent a screening panel of biomarkers according to the existing guidelines. Our findings of high Neu count and high values of CRP, DD, FR, and pro-BNP as risk factors for severe MIS-C (**Table 3**) are comparable to those of several previous studies (10, 26, 34, 36, 38, 43, 44). In one of the largest cohorts of 1,080 MIS-C patients, Abrams et al. determined that the odds of severe MIS-C increased significantly by two times with increased CRP, FR, and DD levels, and MIS-C was five times more likely if the level of pro-BNP was above 2,000 pg/ml (34). A similar importance of pro-BNP was reported in the study performed by Kaidar et al., where an increasing amount of pro-BNP could increase the risk of severe MIS-C by 8.4 times upon reaching levels above 8,000 ng/L (26). The significance of cardiac biomarkers in the early prediction of MIS-C in pediatric patients with COVID-19 has been determined in several studies (44, 45). Gullu et al. identified that a pro-BNP value of 282 ng/L or more alone had a striking sensitivity of 100% and a specificity of 93% with an AUC of 0.985, while an increase in troponin I was less sensitive (60%) but more specific (99.2%, AUC of 0.794) (44). Furthermore, a study from Israel has shown the associations of hemoglobin level ≤95 g/L [odds ratio (OR) 3.356 (1.06–10.61)], PLT count <150 K/ml [OR 4.26 (1.40–12.96)], and CRP value ≥200 mg/L [OR 4.44 (1.45–13.58)] with more severe MIS-C courses (26). We did not find any association of hemoglobin, lymphocyte, and PLT counts with the different severity groups, which was reported earlier (6, 11, 26, 27, 34). Certainly, our cohort was small, and some other studies have addressed higher number of patients. Moreover, we did not include patients older than 18 years of age in our analysis. This population is shown to be associated with more severe MIS-C (46). Nevertheless, in our study, we observed that older patients presented with more severe MIS-C features compared with younger ones [7 (6.9–8.8) vs. 10 (7.6–12.6), respectively, $p = 0.046$]. As expected, all the routine inflammatory markers used for the diagnosis of MIS-C could predict the severity of the condition in our cohort. The combination of CRP and Neu alone was highly specific (92.6%) but was not sensitive enough (60%) to predict the severity group. In addition, the combination of all biomarkers in the MIS-C diagnostic panel showed the highest sensitivity and specificity (**Table 4**). However, not all MIS-C routine biomarkers are freely available in primary care or small regional hospitals (43). Thus, a simpler and more easily obtainable prediction model of disease severity would enable faster referral of suspected severe cases to specialized centers or prompt prescription of specific treatment.

The need for new biomarkers for the evaluation of severe conditions has raised interest in the involvement of PLTs in immunity and their activation signs. Recently, a clinically well-known prognostic factor of thrombocytopenia in severe bacterial infections was supplemented by changes in the MPV and PDW as evidence of innate and adaptive immune response activation (18). Moreover, Barrett et al. found that reticulated PLT count,

larger size, and immaturity in SARS-CoV-2 infection were associated with more severe disease and all-cause mortality (25). Furthermore, in severe cases of MIS-C that needed inotropic support or a longer stay in the PICU, the PLT count was lower and DD and FB levels were higher, indicating upregulated coagulation (6, 26–29, 34). PVT volume index (PVI) values as prognostic indices have been demonstrated in several studies of COVID-19 patients (47); however, data on MIS-C are limited. In a retrospective single-center study of 64 MIS-C patients, Alkan et al. showed that MPV was significantly higher in the most severe patient group (39). However, other parameters (PDW and PCT) could not differentiate between severity groups. In our patient cohort, none of the PLT indices alone differed between the severity groups. MPV and PDW tended to be higher in patients with more severe disease, but the difference was not significant ($p = 0.076$ and $p = 0.068$, respectively). Interestingly, we found that a combination of these biomarkers (PLT, MPV, PCT, and PDW) with a cutoff value of 0.716 could predict MIS-C severity with a sensitivity of 80.8% and specificity of 91.7% (AUC of 0.901; 95% CI of 0.803–0.998; $p < 0.001$), demonstrating the potential of a fast and reliable composite biomarker in clinical practice. A routine clinical blood test using automated hematology analyzers can easily evaluate PVI and count the PCT—the volume occupied by PLTs in the blood as a percentage; thus, it could be easily applied in settings without the possibility of testing biomarkers, such as pro-BNP. PLTs have been shown to have the potential to differentiate between bacterial and viral diseases (48), which could help clinicians to differentiate between sepsis and viral-induced sequelae if MIS-C is suspected. Definitely, further studies are needed. Moreover, more research including healthy children, children with other viral diseases, and children with real KD should be included in a broader analysis to justify the use of PLT markers in clinical practice.

In conclusion, our study is the first in Lithuania to describe clinical and laboratory parameters associated with MIS-C severity, highlighting the significant role of PLTs in MIS-C pathogenesis and severity. PLTs and PLT index measurements in routine laboratory analyses may be helpful in predicting MIS-C severity. However, further prospective and follow-up studies are warranted.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Kaunas Regional Biomedical Research Ethics Committee, Lithuanian University of Health Sciences, Kaunas, Lithuania. Written informed consent to participate in this study from the participants' legal guardian/next of kin was not required in accordance with the national legislation and the institutional requirements.

Author contributions

ASn: original idea; ASn and LJ: writing of original draft and review; LJ: data cleaning, statistical analysis, editing, and supervision; ASi, LV, and RS: data collection and review. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Treatment of immunoglobulin-resistant kawasaki disease: a Bayesian network meta-analysis of different regimens

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Background: This study aimed to gather evidence from clinical trials on the efficacy and safety of the available treatments for intravenous immunoglobulin (IVIG)-resistant Kawasaki disease (KD) in children.
Methods: This work adopted the Newcastle–Ottawa scale to analyse the quality of the enrolled articles. A network meta-analysis was performed using clinical trials that compared drugs used to treat IVIG-resistant KD. Aggregate Data Drug Information System software v.1.16.5 was employed to analyse whether infliximab, second IVIG infusions, and intravenous pulse methylprednisolone (IVMP) were safe and effective.
Results: Ten studies, involving 704 patients with IVIG-resistant KD, were identified and analysed. Overall, infliximab exhibited remarkable antipyretic activity compared with the second IVIG infusions (2.46, 1.00–6.94). According to the drug rank, infliximab was the best option against IVIG-resistant KD. Regarding adverse effects, the infliximab group was more prone to hepatomegaly. A second IVIG infusion was more likely to result in haemolytic anaemia. IVMP treatment was more susceptible to bradycardia, hyperglycaemia, hypertension, and hypothermia. In addition, infliximab, IVMP, and the second IVIG infusions showed no significant differences in the risk of developing a coronary artery aneurysm (CAA).
Conclusion: Infliximab was the best option against IVIG-resistant KD, and IVMP, infliximab, and second IVIG infusions have not significant differences of prevent CAA in patients with IVIG-resistant KD.
Systematic Review Registration: Identifier: https://osf.io/3894y.

KEYWORDS

immunoglobulin, resistant kawasaki disease, infliximab, IVIG, methylprednisolone

Introduction

Kawasaki disease (KD) is an acute, self-limiting, systemic vascular inflammation mainly occurring in small arteries, particularly the coronary arteries (1, 2). In the acute stage, immunoglobulins administered at high doses may decrease coronary artery injury, but 15%–20% of such cases will develop intravenous immunoglobulin (IVIG)-resistant KD (3). According to the literature, coronary artery aneurysm (CAA) incidence is 9-fold higher in IVIG-resistant KD cases than that in IVIG-sensitive cases (4). IVIG-resistant KD may have an increased risk of coronary artery injury compared to IVIG-sensitive KD. Therefore, the risk of coronary artery injury and hospitalisation duration and costs are reduced if IVIG-resistant KD cases are detected, and appropriate treatment is administered prior to further IVIG therapy.

For febrile IVIG-resistant cases, no clear guidelines are available for treatment, which presents a typical challenge. Patients with IVIG-resistant KD should be treated with the

second IVIG infusions (2 g/kg for 1 day). An alternative approach is either 30 mg/kg intravenous pulse methylprednisolone (IVMP; 30 mg/kg for 2–3 h once daily for 3 days) or infliximab (5 mg/kg for 1 day) (5). Infliximab is the drug of choice for treating IVIG-resistant KD (6). However, no uniform treatment guidelines are available, and many different treatments exist among diverse medical centres (7). In addition, drug-related adverse effects (AEs) remain unclear.

Several studies have investigated different drugs for treating IVIG-resistant KD. Previous meta-analyses showed that IVMP and infliximab exhibited higher efficacy than the second IVIG infusions (8, 9). However, the previous pairwise meta-analysis could only analyse two drugs. Network meta-analysis (NMA) can analyse multiple drugs based on clinical research. It has a high reference value for evaluating the advantages of interventions and can provide the best evidence for clinical decision-making. This study aimed to perform a systematic review and Bayesian NMA on paediatric patients reported in studies published in several databases over the past 15 years to investigate the efficacy and safety of different drug regimens for treating IVIG-resistant KD.

Methods

The present study performed NMA and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines extended to NMA (10). Additionally, this study utilised a population-intervention-comparison-outcome framework to include studies describing the treatment of IVIG-resistant KD. Our study protocols were registered in the OSF Registries (https://osf.io/3894y).

Database search

Relevant databases, such as PubMed, Embase, ScienceDirect, ProQuest, ClinicalTrials.gov, ClinicalKey, Cochrane CENTRAL, and Web of Science, were comprehensively searched until 1 May, 2022 to identify relevant studies. The search strategy was approved by the review teams (LH and QF). Regarding the search strategy, the Medical Subject Headings used were (Mucocutaneous Lymph Node Syndrome OR Kawasaki disease) AND (methylprednisolone OR intravenous immunoglobulin/IVIG OR infliximab OR corticosteroids OR steroids OR glucocorticoids OR TNF blockers). No study design or language restrictions were imposed. Additionally, the reference lists of the enrolled studies were manually searched. Finally, two reviewers (LH and QF) reviewed the studies and extracted relevant data.

Study quality assessment

This study used the Newcastle–Ottawa scale (NOS) to assess the quality of the enrolled observational studies. Typically, we judged the NOS statements on three aspects (selection, outcome, and comparability) involving eight items. The Cochrane Collaboration-recommended risk-of-bias approach was used to assess the quality of the randomised clinical trials. A final score of six or more stars was considered high quality.

Selection criteria

Studies conforming to the criteria below were included: (a) patients with the diagnosis of KD in line with the Japanese diagnostic criteria, as well as common standards from the 2017 American Heart Association (i.e., IVIG resistance was defined as persistent or recrudescent fever [T ≥38.0 °C] at least 36 h after completion of the first IVIG infusion), (b) odds ratios (ORs) together with relevant 95% confidence intervals (CIs) regarding categorical variables or numbers and standard deviations could be obtained from the studies, and (c) statistical approaches were clearly described, and statistical analysis was conducted accordingly. The following studies were excluded: (a) studies with defects or low-quality (NOS score<six stars), (b) no ORs or 95% CIs could be obtained for categorical variables, and (c) reviews, duplicates, or unpublished literature.

Statistical analyses

This study utilised NMA to analyse all enrolled articles. Moreover, Aggregate Data Drug Information System software v. 1.16.6 was used to compare the safety and effectiveness of diverse therapeutic agents (11). The Bayesian method was applied in the NMA, which made it possible to compare diverse treatments among different studies (12). We adopted a random-effects model with the Bayesian method through a Markov chain Monte Carlo simulation to obtain the combined effect sizes. We also drew a consistency model to analyse the outcomes assessed and determined the relative effect sizes of treatments based on ORs. Instead of the fixed-effects model, we utilised the random-effects model because it is suitable and conservative for interpreting interstudy variance. Residual deviance was also used to evaluate the goodness of fit of the models. To increase the accuracy of comparison effect sizes and appropriately explain the relationships of multiarm studies, this study constructed rank probabilities that involved every intervention in every outcome to draw conclusions for diverse outcomes of interest (13). Data were expressed with 95% CIs. Subsequently, diverse treatments were ordered based on the highest to the lowest probabilities.

Result

Study selection and description

A total of 101 eligible articles were included (**Figure 1**). Of these, 96 did not meet the inclusion criteria and were not subjected to further examination. We excluded two publications because they did not provide detailed genotypic information.

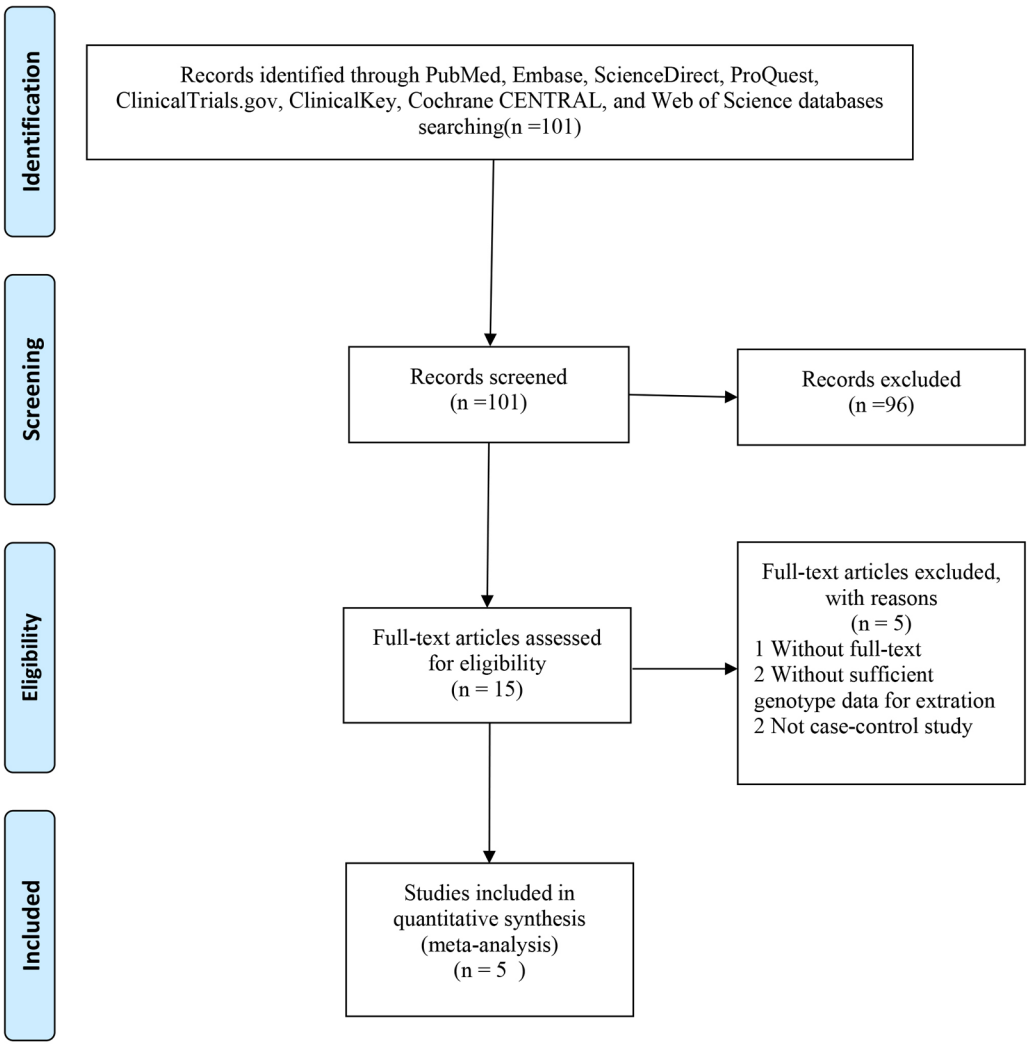

FIGURE 1
The flowchart of the included studies in the meta-analysis.

We also excluded two publications because they were not case-control studies. Furthermore, one publication was removed because the full text was not available. In line with our inclusion and exclusion criteria, this work selected 10 articles published between 2003 and 2021 (13–22). Among these, seven were randomised controlled trials (RCTs) (1, 15, 16, 20–22), and three were non-RCTs (14, 18, 19), as determined based on the Cochrane Handbook. The generation of random sequences was not utilised by Furakawa et al. and Teraguchi et al. since some patients were unwilling to receive IVMP and therefore received a second dose of IVIG (14, 19). Data from Son et al. were collected through a retrospective chart review, and all studies were rated as ≥six stars (high quality) (18). Baseline features on admission were comparable among the diverse treatments, such as age at fever onset, sex, race, duration between fever onset and diagnosis, and duration from the first treatment to retreatment. Five studies were conducted in Japan, three in America, and one each in China and Korea (**Table 1**).

Antipyretic effects

Infliximab was associated with significant antipyretic effects compared with the second IVIG infusion (2.46, 1.00–6.94, **Figure 2**). No significant differences were recorded between the IVMP and IVIG retreatment groups (0.92, 0.25–3.51). Furthermore, no significant differences were recorded between infliximab and IVMP (2.70, 0.53–14.42). According to the drug rankings (see **Figure 3**), infliximab had better antipyretic effects than the other drugs. Based on the current research results, infliximab is the best option against IVIG-resistant KD.

AEs

All included studies reported AEs during the disease course, except for the study by Teraguchi et al. (**Tables 2**, **3**). In

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TABLE 1 Study selection and subject characteristics of included studies in meta-analysis.

Author	Year	Country	Design	Patients		Initial treatment	Retreatment		Antipyretic effects		CAA		NOS
				Cases	Controls		Cases	Controls	Cases	Controls	Cases	Controls	
Son	2011	US	Non-RCT	20	86	IVIG	Infliximab	IVIG	17	71	/	/	6
Tremoulet	2014	US	RCT	98	97	IVIG	Infliximab	IVIG	87	86	25	20	9
Youn	2016	Korea	RCT	11	32	IVIG	Infliximab	IVIG	10	21	1	4	6
Masaaki	2018	Japan	RCT	16	15	IVIG	Infliximab	IVIG	12	5	1	3	8
Burns	2021	US	RCT	54	49	IVIG	Infliximab	IVIG	42	25	4	1	9
Miura	2008	Japan	RCT	7	8	IVIG	IVMP	IVIG	4	4	2	2	8
Furukawa	2008	Japan	Non-RCT	44	19	IVIG	IVMP	IVIG	34	12	5	2	6
Ogata	2009	Japan	RCT	13	14	IVIG	IVMP	IVIG	13	14	0	3	8
Teraguchi	2013	Japan	Non-RCT	14	27	IVIG	IVMP	IVIG	7	21	5	7	6
Wang	2020	China	RCT	40	40	IVIG	IVMP	IVIG	40	40	18	15	9

RCT, randomized controlled trial; NOS, newcastle-ottawa scale; CAA, coronary artery aneurysm.

summary, hepatomegaly was more likely to occur in the infliximab group. Patients undergoing a second IVIG treatment were more likely to develop haemolytic anaemia. Compared with the second IVIG infusion, IVMP treatment was more susceptible to bradycardia, hyperglycaemia, hypertension, and hypothermia.

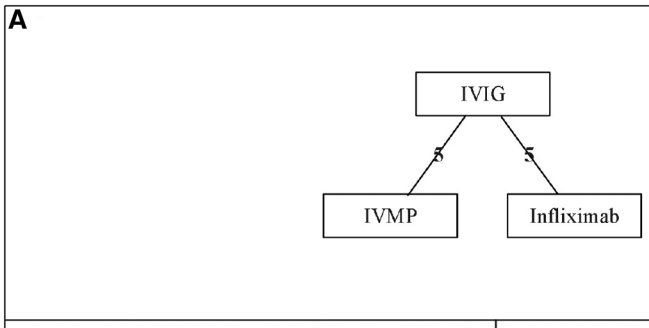
CAA

All included studies reported CAA, except for the study by Son et al. There were no significant differences in the risk of CAA between infliximab and the second IVIG infusion (1.34, 0.45–4.08). No significant differences were recorded between the IVMP and IVIG-retreatment groups (1.00, 0.25–2.91). Furthermore, no significant differences were observed between infliximab and IVMP (1.37, 0.31–8.29).

Discussion

As reported in a multicentre study, IVIG-resistant cases may have CAA (18.6%), although the first adequate IVIG treatment can reduce IVIG nonresponse (23). Some optimal clinical treatments have been proposed to manage IVIG-resistant cases, among which a second IVIG infusion alone or in combination with corticosteroids, long-course corticosteroids alone, or infliximab plus pulsed therapy has been frequently selected. Continuous or relapsed fever following the initial IVIG dose, but not laboratory measurements of inflammation, has been recognised as an indicator of continuous inflammation. A consensus has been reached that additional treatments should be administered to patients with such symptoms. According to the KD guidelines of the American Heart Association (AHA), a second IVIG dose or steroid therapy is assigned a B evidence level (non-RCTs), whereas infliximab is assigned a C evidence level (expert consensus) (24). The International Society for Pharmacoeconomics and Outcome Research recommends using NMA to compare outcomes among diverse treatment modalities. Thus, a robust NMA is required to guide treatment.

Previous meta-analyses have shown that IVMP and infliximab treatments are more effective than the second IVIG dose in terms of fever resistance (8, 9). Our NMA comparing the three drugs, showed that infliximab was the best option for treating IVIG-resistant KD. The expression of tumour necrosis factor (TNF)-α increases among patients with acute KD, with the greatest expression being observed in patients developing CAA (24). TNF inhibitors can mitigate endarteritis and inflammation by inhibiting the adhesion of neutrophils onto endothelial cells (ECs). Infliximab, the anti-TNF-α chimeric monoclonal antibody, has been used to treat IVIG-resistant KD over the last decade. In retrospective studies from two institutions, IVIG-resistant KD cases receiving infliximab as initial retreatment showed markedly rapid fever resolution and shortened length of stay (LOS) relative to those receiving IVIG (17). Another study



B Efficacy outcome

Consistency Analysis		
IVIG	0.92(0.25,3.51)	2.46(1.00,6.94)
	IVMP	2.70(0.53,14.42)
		Infliximab

C Ranking of efficacy outcome

Drugs	Rank 1	Rank 2	Rank 3
IVIG	0.01	**0.55**	0.43
IVMP	0.10	0.35	**0.55**
Infliximab	**0.89**	0.10	0.01

FIGURE 2
(A) Network of comparisons for efficacy outcome. The nodes (drugs) are represented by circles. The grey circles represent stimulant drug and the white circles represent non-stimulant drugs. The lines connecting each drug represent direct comparisons, while indirect ones were statistically estimated. The thickness of the line represents the amount of existing comparisons and the size of the circles (nodes) indicates the sample-size number. (B) Consistency analysis for the outcome of efcacy. Drugs are reported in alphabetical order. The values presented correspond to the mean diference (MD) associated with its credibility interval (CrI). When the CrI does not cross the 0 null line, there is a statistically signifcant diference between the treatments. Comparisons are made between a first drug (e.g. IVIG) and a second drug (e.g. IVMP) with presentation of the estimated value (0.92 [0.25-3.51]). An MD value of less than 0 demonstrates that the frst drug in the comparison is the more effective. An MD value greater than 0 indicates that the second drug in the comparison is more efective. The highlighted pictures presented statistical diferences. IVIG, intravenous immunoglobulin; IVMP, intravenous pulse methylprednisolone. (C) Rank probabilities of drugs. The values are given as the probability of each treatment occupying a position. Ranking 1 is the best therapy and the last one is the worst treatment for this outcome. IVIG, intravenous immunoglobulin; IVMP, intravenous pulse methylprednisolone.

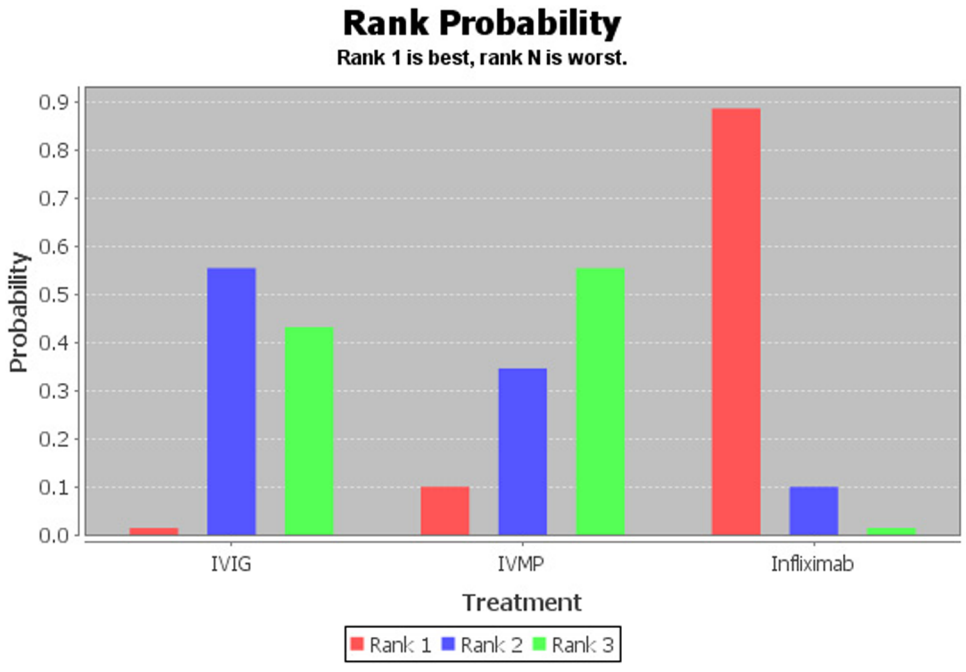


FIGURE 3
Rank probability graph of drugs. The values are given as the probability of each treatment occupying a position. Ranking 1 is the best therapy (more likely to lead to antipyretic effects) and the last one is the worst treatment for this outcome.

in 2021 compared the cost-effectiveness between infliximab and a second IVIG infusion in IVIG-resistant cases; according to the results, for 100 IVIG-resistant cases receiving 10 mg/kg infliximab treatment, US$ 824,759 was saved (25). Such decreased costs were related to a reduction in cost/dose and infusion duration, and 24-h monitoring prior to discharge,

TABLE 2 The incidence of AEs in the included studies (infliximab VS. IVIG).

AE description	Son		Tremoulet		Youn		Masaaki		Burns	
	Infliximab	IVIG	Infliximab	IVIG	Infliximab	IVIG	Infliximab	IVIG	Infliximab	IVIG
Hemolytic anemia	/	/	2	1	/	/	/	/	19	19
GI symptoms	/	/	/	/	/	/	2	3	3	4
Rash	/	/	/	/	/	/	3	0	2	5
Epistaxis	/	/	/	/	/	/	4	7	2	3
Infusion Reaction	/	/	/	/	0	5	/	/	2	1
Arthritis	/	/	/	/	/	/	/	/	2	4
Headache	/	/	2	1	/	/	/	/	1	2
URI	/	/	/	/	/	/	3	2	1	2
Hepatomegaly	6	1	/	/	/	/	/	/	/	/

URI, upper respiratory tract infammation; GI, gastro-intestinal; AEs, adverse effects; IVIG: intravenous immunoglobulin.

TABLE 3 The incidence of AEs in the included studies (IVMP VS. IVIG).

AE description	Miura		Furukawa		Ogata		Teraguchi		Wang	
	IVMP	IVIG	IVMP	IVIG	IVMP	IVIG	IVMP	IVIG	IVMP	IVIG
Bradycardia	6	2	3	0	2	0	/	/	5	0
Hyperglycemia	5	0	/	/	/	/	/	/	/	/
Hypertension	6	5	5	0	/	/	/	/	/	/
Hypothermia	/	/	3	0	/	/	/	/	/	/

AEs, adverse effects; IVIG: intravenous immunoglobulin; IVMP, intravenous pulse methylprednisolone.

which shortened the LOS (14). Therefore, our study further confirmed the potential value of infliximab treatment in patients with IVIG-resistant KD. These results could be conducive to recommending an objective order of these treatment options in future studies and guidelines.

In severe KD cases, cardiovascular complications or manifestations have been strongly associated with the incidence and mortality in the acute phase or during long-term follow-up. As revealed by Millar et al., corticosteroid application in acute KD patients who developed CAA possibly induced aggravation of aneurysms, as well as impairment of vascular remodelling (15). As reported by the AHA, steroids only apply to paediatric patients who do not respond to ≥two IVIG infusions for treating continuous fever (26). However, according to previous meta-analyses, infliximab, second IVIG, and IVMP were not significantly different in CAA prevention (8, 9). Similar results were obtained in this study. These drugs may suppress cytokine generation, which is important for reconstructing the affected coronary artery wall (17). It is necessary to further investigate the long-term coronary artery outcomes among treated KD cases and to estimate coronary artery endothelium function in KD cases.

Certain limitations should be noted in this work. First, many articles included in this study were observational RCTs, which may have led to an increased risk of heterogeneity. Second, only infliximab (a TNF inhibitor) was used in every enrolled study, making it impossible to assess the efficacy of additional TNF inhibitors in IVIG-resistant KD. Third, our enrolled articles were collected from published literature, and some unpublished articles might have been missed. Finally, although no significant statistical or clinical heterogeneity was observed across the included studies, potential bias existed because the literature is limited. Most included studies did not completely evaluate the postretreatment incidence of CAAs in patients with IVIG-resistant KD after short-term follow-up. Therefore, large, homogeneous, randomised clinical trials with long follow-up periods are required.

Infliximab was the best options against IVIG-resistant KD, respectively. In addition, IVMP, infliximab and second IVIG infusion have not significant differences of prevent CAA in IVIG-resistant KD patients. More studies will need to be conducted to evaluate the different drug regimens of IVIG-resistant KD.

Data availability statement

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

Author contributions

YP contributed to the design of the study and reviewed and revised the manuscript. YP followed up with the patient, collected information from the literature, and wrote sections of the manuscript. YP provided assistance for treatment. YP collected the data and followed up with the patient. YP approved the final manuscript as submitted and agree to be accountable

for all aspects of the work. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Prognostic role of euthyroid sick syndrome in MIS-C: results from a single-center observational study

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Background: Euthyroid sick syndrome (ESS) is characterized by low serum levels of free triiodothyronine (fT3) with normal or low levels of thyroid stimulating hormone (TSH) and free thyroxine (fT4) and is reported in different acute clinical situations, such as sepsis, diabetic ketoacidosis and after cardiac surgery. Our aim was to evaluate the predicting role of ESS for disease severity in patients with Multisystem Inflammatory Syndrome in children (MIS-C).
Methods: A single-centre observational study on consecutive patients with MIS-C. Before treatment clinical, and laboratory data were collected and, in a subset of patients, thyroid function tests were repeated 4 weeks later. Variables distribution was analyzed by Mann-Whitney *U*-test and correlations between different parameters were calculated by Spearman's Rho coefficient.
Results: Forty-two patients were included and 36 (85.7%) presented ESS. fT3 values were significantly lower in patients requiring intensive care, a strong direct correlation was shown between fT3 and Hb, platelet count and ejection fraction values. A significant inverse correlation was retrieved between fT3 levels and C-reactive protein, brain natriuretic peptide, IL-2 soluble receptor and S-100 protein. Subjects with severe myocardial depression (EF < 45%) had lower fT3 values than subjects with higher EF. The thyroid function tests spontaneously normalized in all subjects who repeated measurement 4 weeks after admission.
Conclusion: ESS is a frequent and transient condition in acute phase of MIS-C. A severe reduction of fT3 must be considered as important prognostic factor for severe disease course, with subsequent relevant clinical impact in the management of these patients.

KEYWORDS

children, COVID-19, euthyroid sick syndrome, multisystem inflammatory syndrome, SARS-CoV2

Abbreviations

ACE, angiotensin-converting enzyme; BNP, brain natriuretic peptide; cAMP, cyclic adenosine monophosphate; CBC, complete blood count; CDC, Centers for Disease Control and Prevention; CNS, Central nervous system; Covid-19, CoronaVirus disease 2019; CRP, C-reactive protein; D1, deiodinase 1; D3, deiodinase 3; EF, ejection fraction; EKG, electrocardiogram; ESR, erythrosedimentation rate; ESS, euthyroid sick syndrome; fT3, free triiodothyronine; fT4, free thyroxine; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase; Hb, haemoglobin; ICU, intensive care unit; IFN, interferon; IL, interleukin; IL-2R, soluble IL2 receptor; MIS-C, Multisystem Inflammatory Syndrome in children; PFAPA, periodic fever, aphtous stomatitis, pharyngitis, adenitis; rT3, reverse T3; S100, S-100 protein; SARS-CoV2, Severe Acute Respiratory Syndrome –CoronaVirus 2; Tg, thyroglobulin; TNF, tumor necrosis factor; TnI, troponin I; TSH, thyroid stimulating hormone; WBC, white blood cells.

Introduction

From May 2020, after the first reported cases of "Kawasaki-like disease" (1), a novel multisystem inflammatory syndrome in children (MIS-C) related to COVID19 infection was identified. It was defined by signs of systemic inflammation (fever, elevated inflammatory markers) and at least two organ dysfunctions in an individual aged <21 years, with current infection or exposed within 4 weeks to SARS-CoV2 (2). The clinical manifestations of MIS-C are very heterogeneous but cardiovascular and gastrointestinal systems appear to be the most affected (3). Few studies have explored possible manifestations affecting the endocrine system during this iperinflammatory disease related to SARS-CoV2.

Recently, an alteration of the thyroid axis known as *euthyroid sick syndrome* (ESS) has been reported in patients with MIS-C (4). This condition, described in children with severe clinical situations such as sepsis (5), diabetic ketoacidosis (6) or after cardiac surgery (7), is characterized by decreased levels of triiodothyronine (fT3), increased conversion of thyroxine (fT4) to the biologically inactive form of reverse T3 (rT3) without a compensatory rise in of thyroid-stimulating hormone (TSH) (8). The result is an overall reduction in the bioavailability of active fT3, thus miming central hypothyroidism (9).

ESS appears as an adaptive mechanism to reduce the energy expenditure of the organism. For this reason, there is no agreement to correct this condition although the severity of ESS is associated with poor outcome in some studies (10).

In this study, our aim was to collect and analyze the thyroid profile together with other laboratory and instrumental data in order to evaluate the possible prognostic role of ESS within a cohort of patients affected by MIS-C.

Patients and methods

Patients

A single-center observational study was conducted at the Pediatric Rheumatology Unit of the Department of Woman and Child Health of Padova between 30 November 2020 and 30 September 2022. Consecutive patients admitted for MIS-C, according to classification criteria proposed by the Center for Disease Control and Prevention (CDC), were included (3). Children with known thyroid, hypothalamic and pituitary disease or undergoing medical therapy with glucocorticoids or inotropes in the 48 h prior to admission were excluded. This study was approved by the Ethical Committees of Padova University Hospital (n. 338n/AO/23). Due to the nature of the study (observational cross-sectional), no informed consent was required from the patients and their caregivers.

Clinical data collection

Laboratory tests were performed for each patient upon admission and before any treatment. More precisely, collected data were: complete blood count (CBC), C-Reactive Protein (CRP), Erythrosedimentation Rate (ESR), ferritin, cytokines and biomarkers profile (interleukins IL-1α, IL-1β, IL-6, soluble IL2 receptor, IL2r, tumor necrosis factor α, TNF α, and S-100 protein), organ involvement indices such as glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), troponin (TnI), brain natriuretic peptide (BNP), thyroid hormones (TSH, fT3, fT4).

Thyroid function tests were considered normal according to the range values of the hospital laboratory: TSH 0.3–5 mU/L; fT3 3.9–6.8 pmol/L; fT4 8–20 pmol/L.

A complete cardiological evaluation including Electrocardiogram (EKG) and echocardiography was performed upon admission. In a group of patients, a second dosage of TSH, fT3 and fT4 levels was performed 4 weeks after the onset of the disease.

Statistical analysis

The continuous variables were collected by calculating the main indicators of centrality and variability. The analysis of the differences in demographic and clinical features and in laboratory values between groups of subjects defined according to variables of interest was made by applying the non-parametric Mann-Whitney *U*-test, after verifying the non normality of the distributions of the variables under examination.

Fisher's exact test or the χ^2 test were used for group comparison (ESS group vs. no ESS group). Spearman's Rho correlation coefficient was calculated to evaluate the correlation between the different laboratory values. A p-value less than 0.05 (two-tailed test) was considered statistically significant.

Post-hoc power analysis was calculated on the fT3 values variable (two-tailed hypothesis) given the observed probability level (0.05), the observed effect size (Hedges'g = 2.4202) and the total sample size. Hedges'g was chosen because more appropriate where very different sample sizes are considered, as in the present study. All analyses was performed using IBM SPSS statistical software. (Vers. 20.0).

Results

Demographic, clinical and laboratory features of MIS-C patients at onset

In the study we included 42 consecutive children with MIS-C (29 male—69%) with median age 9 years (range 0.7–17 years). As showed in **Table 1** the most represented ethnicity was Caucasian (88%), although children of African descent appeared more often involved (9.5%) compared to other ethnic origin. Only 4 subjects had comorbidities [1 periodic fever with periodic fever, aphtous stomatitis, pharyngitis, adenitis (PFAPA syndrome), 2 glucose 6-phosphate dehydrogenase deficiency, 1 autism spectrum disorder]. According to case definition of MIS-C, all patients were exposed to SARS-CoV2. Thirty patients (71%) reported previous confirmed infection with a median time of 4 weeks (range 2–6 weeks).

TABLE 1 Laboratory tests of whole MIS-C group and comparison between patients with ESS and patients with normal thyroid function tests (no ESS group) at the onset of disease.

Variable	Overall ($n = 42$) Median (range)	ESS ($n = 36$) Median (range)	No ESS ($n = 6$) Median (range)	p value
White Blood Cells ($\times 10^9$/L)	8.26 (3.03–27.68)	8.53 (3.03–27.68)	7.94 (4.53–12.90)	0.49
Lymphocytes ($\times 10^9$/L)	1.40 (0.41–3.30)	1.43 (0.42–3.30)	935 (0.41–3.04)	0.38
Hemoglobin (g/dl)	11.4 (7.4–13.9)	11.4 (7.4–13.9)	12.3 (10.3–13.7)	0.25
Platelets ($\times 10^9$/L)	159.00 (52.00–449.00)	155.50 (52.00–449.00)	255.50 (127.00–434.00)	**0.03**
CRP (mg/L)	137 (19.1–546)	163 (47–546)	111 (19.1–152)	**0.04**
GOT (U/L)	35 (21–221)	35 (21–221)	63 (30–137)	0.24
GPT (U/L)	27 (12–97)	28 (13–97)	28 (12–57)	0.32
Ferritin (ug/L)	550 (121–2,905)	738 (159–2,905)	294 (121–416)	**0.02**
Troponin I (ng/L)	48 (1.9–29,691)	48 (1.9–1,484)	219 (1.9–29,691)	0.83
Brain natriuretic peptide (ng/L)	202 (8.9–3,731)	211 (9.0–3,731)	82 (9.0–344)	0.14
IL-1α (ng/L)	1.9 (1.9–170)	1.9 (2.0–170)	1.9 (1.9–116)	0.94
IL-1β (ng/L)	7 (3.7–47)	7.2 (4.0–48)	5.6 (3.7–46.5)	0.47
IL-6 (ng/L)	32 (0.5–680)	30 (1.0–680)	79 (9.9–608)	0.26
TNFα (ng/L)	21 (6–75)	21,6 (11–70)	12 (6.3–75.7)	0.10
IL-2r (kU/L)	3,942 (1,190–16,500)	4,452 (1,634–16,500)	1,558 (1,190–2,519)	**0.00**
S-100 protein (ug/L)	0.27 (0.03–1.83)	0.29 (0.05–1.83)	0.14 (0.03–0.39)	**0.02**
TSH (mU/L)	1.58 (0.18–4.37)	1.50 (0.18–3.7)	2.70 (1.22–4.37)	0.06
fT3 (pmol/L)	2.55 (0.96–5.36)	2.39 (1.0–3.9)	4.20 (3.94–5.36)	**0.00**
fT4 (pmol/L)	15.5 (7.3–20.7)	14.7 (7.3–20.7)	17.6 (13.3–18.1)	**0.05**

Bold values are statistically significant.

Forty-one patients (98%) were treated with immunosuppressive therapy using high-dose immunoglobulin (2 g/kg) plus corticosteroid (methylprednisolone 2 mg/kg). Only one subject was treated only with corticosteroid monotherapy. Due to the severity of the disease onset, six subjects (14%) required treatment with interleukin 1 antagonist (Anakinra). The median time between onset of symptoms and initiation of therapy was 6 days (range 2–9 days).

In **Supplementary Table S1**, clinical manifestation at onset are reported. Gastrointestinal tract involvement was the most frequent, affecting 39 patients (93%): reported symptoms were mainly abdominal pain (67%), vomiting (45%) or diarrhea (40%). The involvement of cardiovascular system was observed in 34 patients (81%) presenting with severe arterial hypotension in 14 patients (33%), increased myocardial enzymes in 23 patients (54%), decreased cardiac contractility (Ejection fraction EF < 55%) in 16 (38%), or coronary artery abnormalities (coronary ectasias/aneurysms, coronaritis) in 7 (16%). Sixty-seven percent of individuals presented skin and mucosal involvement. Renal and Central Nervous System (CNS) involvement appeared less frequent, involving only 8 (19%). and 4 children (9%), respectively.

No statistically significant difference was found in clinical features between the ESS and no ESS group (**Supplementary Table S1**).

As shown in **Table 1**, on admission, 36 children (86%) presented ESS characterized by low fT3 levels (median 2.54 pmol/L, range 0.96–5.36): only one subject presented simultaneously low fT4 levels and another had reduced TSH levels. The post-hoc power analysis showed a very high value (0.9999) although the samples size (ESS group and no-ESS group) were very different.

Other relevant laboratory tests showed increased acute phase reactants such as CRP median 137 mg/L (range 19.1–546) and ferritin median 550 ug/L (range 121–2,905) associated with widely variable range of myocardial enzymes alterations as troponin median 48 ng/L (range 1.9–29,691) and BNP median 202 ng/L (range 8.9–3,731).

At disease onset patients in the ESS group showed a higher inflammatory profile compared with euthyroid subjects. In fact, as showed in **Table 1**, a statistically significant elevation of the main inflammatory markers such as CRP ($p = 0.03$), ferritin ($p = 0.02$), IL-2r ($p = 0.003$), and S-100 protein ($p = 0.02$) was observed in ESS patients compared with euthyroid ones. The difference in terms of platelet count ($p = 0.03$) also appeared significant.

In 16 subjects (2 of them admitted in ICU) the measurement of thyroid function tests was repeated 4 weeks after admission and showed normalization in all (TSH mean 2.57 mU/L, range 0.96–5.36 mU/L, fT3 mean 6,07 pmol/L range 5,2–6,7 pmol/L, fT4 mean 19,2 pmol/L range 15,1–20,1 pmol/L.

ESS and risk of intensive care support

Among MIS-C patients, 6/42 (14%) required admission to intensive care unit (ICU): these subjects documented much lower fT3 levels compared to other patients (median value 1.45 pmol/L vs. 2.76 pmol/L, $p = 0.013$), while no significant difference was observed for fT4 and TSH levels (**Figure 1**). Indeed, the subjects admitted to ICU, presented significantly higher inflammatory markers and white blood cells count: respectively WBC (median value 11,355/mmq vs. 8,060/mmq, $p = 0.019$), CRP (median value 229 mg/L vs. 143 mg/L, $p = 0.008$), ferritin (median value 1,383 ug/L vs. 467 ug/L, $p = 0.034$), BNP (median value 1,363 ng/L vs. 187 ng/L $p = 0.004$) and S100 protein (median value 1.08 ug/L vs. 0.24 ug/L, $p = 0.002$).

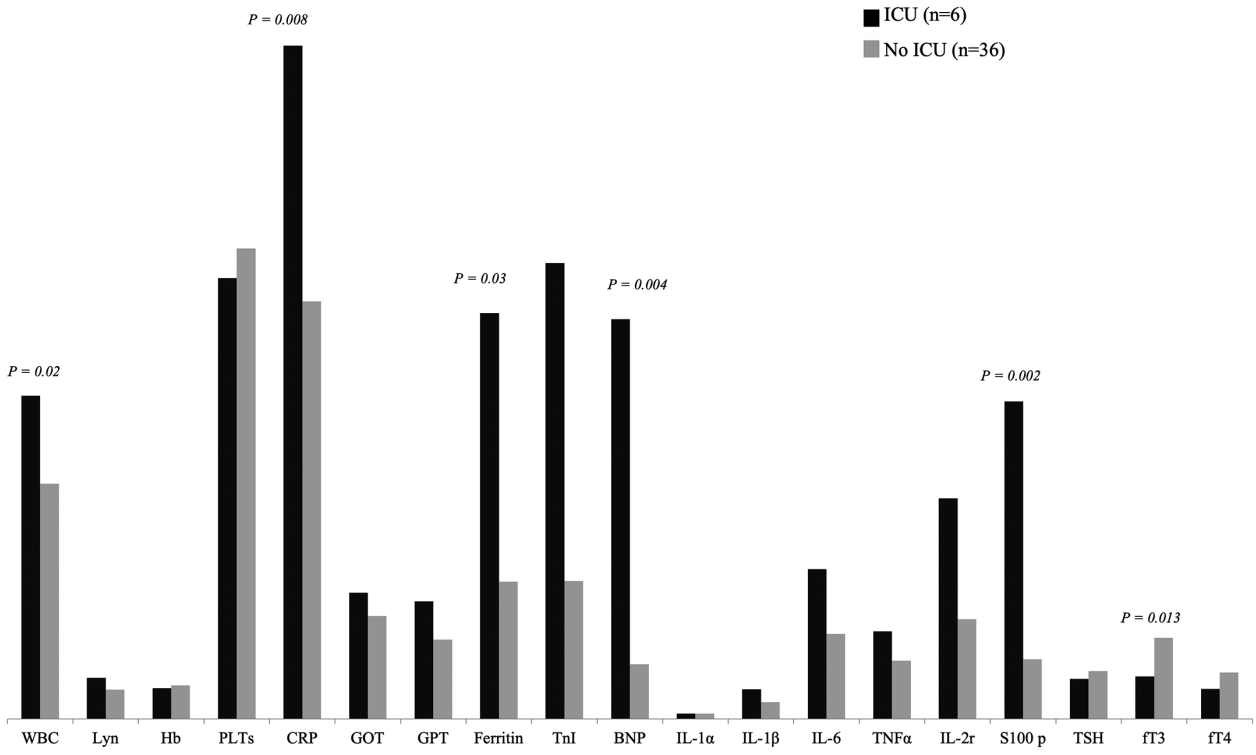

FIGURE 1
Comparison of laboratory features between patients requiring admission to ICU and patients admitted to general hospital ward.

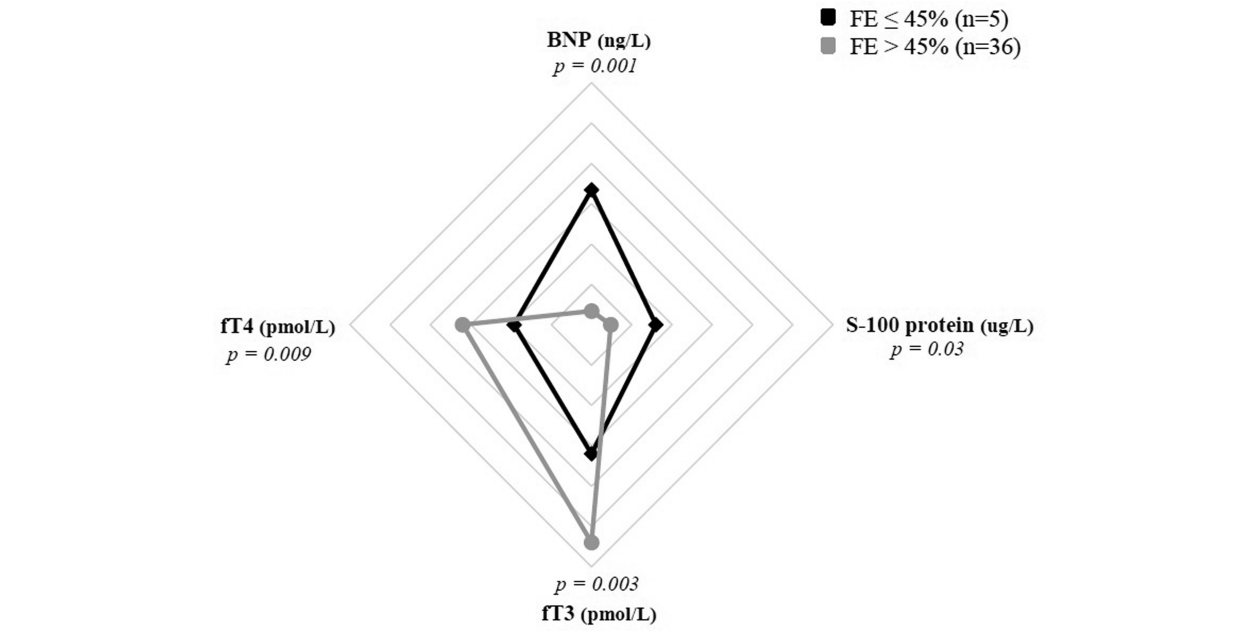

FIGURE 2
Correlation between severity of myocardial dysfunction and ESS.

ESS and risk of severe cardiac involvement

Severe depression of myocardial function, defined by EF ≤ 45%, was found in 5 patients (12%) of MIS-C cohort. This subgroup presented significantly lower fT3 values (median 1.55 pmol/L vs. 2.73 pmol/L; $p = 0.003$) and fT4 (median 9.64 pmol/L vs. 16.02 pmol/L; $p = 0.009$) if compared with EF > 45% subgroup, as showed in **Figure 2**. Conversely, no clear

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association between fT3 values and presence of coronary dilatation at the onset of the disease has been detected.

Prognostic role of ESS for severe clinical course

As shown in Figure 3A,B, statistical analysis demonstrated a direct correlation between ESS severity, expressed by fT3 values, and hemoglobin levels [correlation coefficient (ρ) 0.39, $p = 0.01$] or platelet count (ρ 0.44, $p = 0.003$). An inverse correlation was documented between fT3 levels and markers of systemic inflammation such as CRP (ρ 0.390, $p = 0.009$), soluble IL2 receptor (ρ 0.59, $p = 0.001$) and S-100 protein (ρ 0.39, $p = 0.002$) (Figure 3C,E,F). Moreover, a statistically significant inverse correlation was observed between triiodothyronine and BNP (ρ 0.49, $p = 0.002$) (panel D, Figure 3). About instrumental data, there was a direct correlation between fT3 levels and EF values

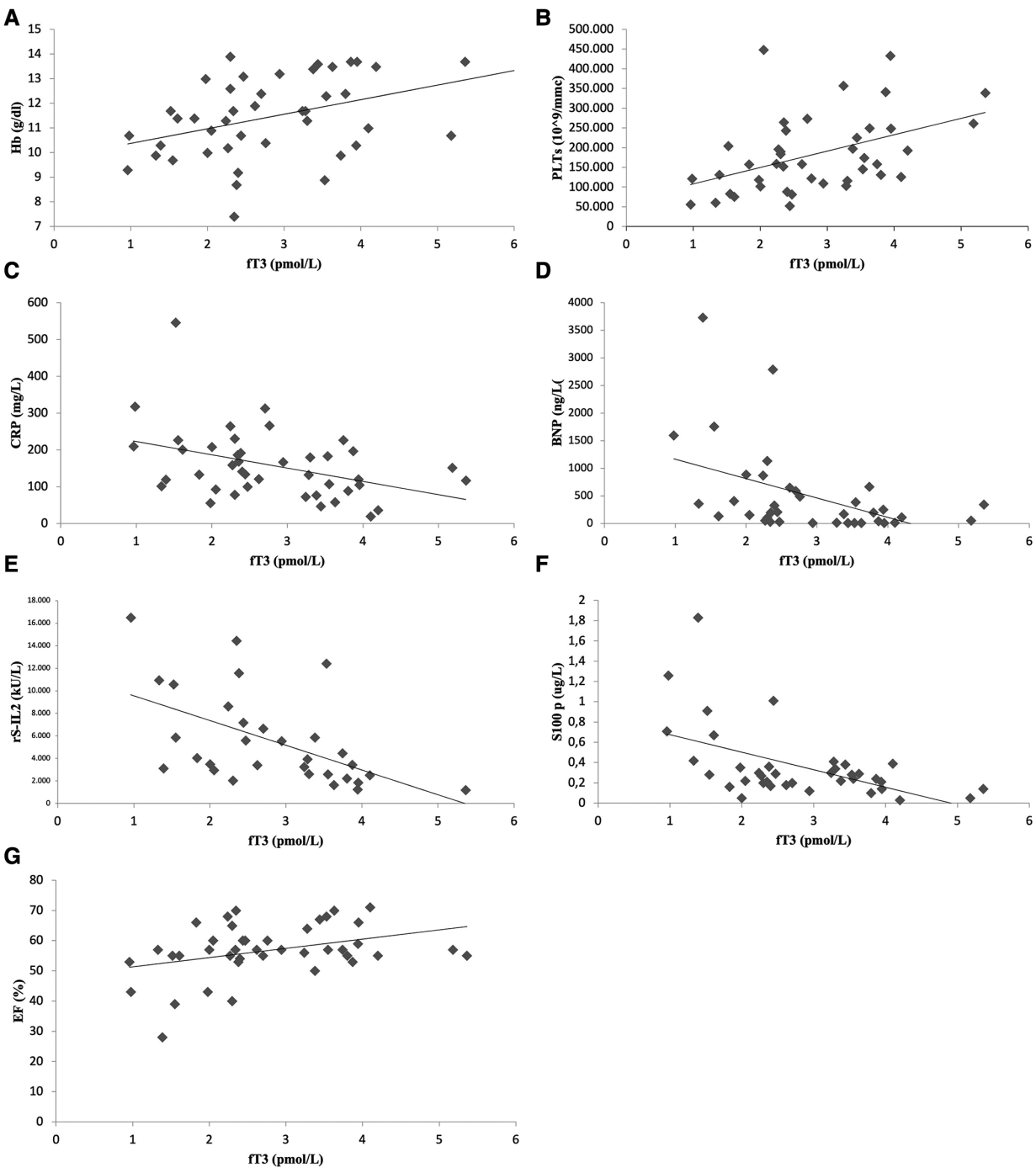

FIGURE 3
Significant correlation between laboratory-instrumental values and fT3 level: scatterplots show correlation between fT3 and hemoglobin (A), platelets (B), C-reactive protein (C), brain natriuretic peptide (D), soluble IL-2 receptor (E), S -100 protein (F) and ejection fraction (G).

on admission (ρ 0.36, $p = 0.017$), as shown in panel F of **Figure 3**. No statistically significant correlation between fT3 values and fever duration at the time of initiation of therapy, WBC (ρ −0.26, $p = 0.09$), lymphocytes count (ρ −0.16, $p = 0.33$), GPT (ρ −0.16, $p = 0.3$), GOT (ρ 0.10, $p = 0.5$), ferritin (ρ −0.15, $p = 0.39$), IL-1α (ρ −0.18, $p = 0.26$), IL-1β (ρ −0.26, $p = 0.11$), IL-6 (ρ 0.12, $p = 0.45$), TNF (ρ −0.33, $p = 0.055$), TnI levels (ρ −0.03, $p = 0.82$).

Discussion

During the COVID19 pandemic, thyroid function alterations have been reported in patients not previously diagnosed with any thyroid conditions. The mechanisms underlying COVID-19-related dysfunction of endocrine glands consist of inflammation, vessel damage, necrosis, degeneration, immune and autoimmune processes (11–14). For the abnormalities of the hypothalamus-pituitary-thyroid axis associated with COVID19 several mechanisms have been suggested such as a disturbance in the TSH process via virus-related hypophysitis, or a subacute thyroiditis linked to the virus spread (11, 15). Subacute thyroiditis during COVID19 presents with thyroid hormone flare-up, usually is self-limited and does not require specific treatment (16, 17). Two major pathophysiological models have been implicated: a direct infection of thyroid cells by SARS-CoV-2 as they express Angiotensin-converting enzyme (ACE) 2 or an indirect effect caused by an immune-inflammatory abnormal response to the virus, especially at the moment of cytokine storm, in addition to multiple organ failure (18–21).

In patients with COVID19 other thyroid function parameters alterations, which are commonly referred as euthyroid sick syndrome (ESS) have been reported (22). ESS has been recognized since the 1970s when it was observed that acute illnesses and fasting may affect circulating levels of thyroid hormones in subjects without previously diagnosed thyroid disease. Most typically, in ESS plasma concentrations of fT3 decrease and those of rT3, the biologically inactive form of fT4, rise (23, 24). This suggests an inactivation of thyroid hormone in peripheral tissues likely mediated by activation of type 3 deiodinase (D3) or by suppressed activity of type 1 deiodinase (D1) (25, 26). As the severity and the length of ESS increases, also the fT4 levels can be deeply reduced (27).

ESS has been reported both in adult and pediatric populations in many severe clinical conditions such as sepsis, trauma, acute myocardial infarction, severe malnutrition, liver failure, cardiac surgery, and diabetic ketoacidosis (5–9). Zou et al. studied 149 COVID19 patients and found that those with ESS (27.5%) had more severe inflammatory responses, such as higher levels of C-reactive protein and erythrocyte sedimentation rate compared to those without ESS (22). In a cohort of subjects with acute COVID19 infection, the presence of ESS correlated with a more severe course of the disease, with higher inflammatory parameters level and risk of myocardial dysfunction compared with normal thyroid profile (28).

In pediatric age, beside a low rate of hospitalization and need for intensive support associated with direct SARS-CoV2 infection, a great burden of morbidity was observed because of the novel multisystem inflammatory syndrome in children (MIS-C) related to COVID19 (1, 2).

MIS-C is an immune-mediated disease that can manifest with multiple organ failure and even lead to shock. The etiology of this syndrome is not yet fully understood: host genetic factors associated to alterations of innate and adaptive immune system as well as mechanisms of molecular mimicry (29) can develop MIS-C as a result of a cytokine storm after the infection (30, 31).

The clinical features and the involvement of internal organs in MIS-C have been extensively studied while only few data are available on dysfunction of endocrine systems and its possible clinical relevance. Calcaterra et al. documented that 23/26 patients (88%) with MIS-C presented ESS at onset predominantly characterized by low fT3 values (65%) as compared to alterations of other thyroid hormones (4). Similarly, in a case-control study, lower fT3 levels were found in patients with MIS-C compared with healthy subjects with opposite results for fT4 levels (32).

In the present study, we assessed the thyroid function in patients with MIS-C and analyzed the potential value of ESS in predicting disease severity. In our series, similarly to previous studies, ESS was present in the majority of patients (86%) with an hormonal profile mainly characterized by reduced fT3 values. In our cohort we did not find statistically significant differences in clinical features between patients with ESS and those with normal thyroid levels, probably due to the small number of patients. Nevertheless, it is worth to note that signs of more severe course such as hypotension, coronary abnormalities, CNS and renal involvement were more frequent in patients with ESS, and this was confirmed by the observation that children requiring admission in intensive care unit were all from the ESS group.

Furthermore, our patients with ESS showed markedly more elevated inflammatory markers such as CRP, ferritin, rsIL-2 and S-100 protein and we observed a strong correlation between fT3 levels and different inflammatory markers while they did not correlate with the duration of the disease. These results suggest that the hyperinflammatory state could be principal responsible for the hormone dysfunction and related to its severity.

This evidence suggests ESS as a useful adaptation of the body to counteract excessive catabolism during illness and as a part of the acute phase response mediated by cytokines (33). In fact, proinflammatory cytokines, especially IL-1, IL-6, TNF-α, and interferon-γ, inhibit several genes involved in thyroid hormone metabolism *in vitro* (34, 35). The administration of cytokines in experimental models resulted in altered thyroid hormone metabolism exhibiting some, but not all, features of disease-related ESS. A possible causal role for IL-6 in the development of ESS was suggested since IL-6 knock out mice showed a less pronounced drop in serum T3 during illness (36). However, acute injection of cytokines induced a flu-like illness but failed to induce ESS like features, except for interferon gamma (IFN-γ) which reduced serum T3 and T4, therefore suggesting that the resulting illness, rather than the cytokines alone, accounted for the changes in thyroid hormones metabolism (37). Other studies showed that several components of the thyroid hormones synthesis pathway can be downregulated by cytokines directly on

the level of the thyrocyte, ultimately leading to decreased secretion of T4 and T3 (38). Moreover, studies *in vitro* and in animal models showed that IL-1α and IL-1b may display multiple effect such as inhibition of the thyroglobulin (Tg) mRNA expression induced by TSH and subsequent Tg release in human cultured thyrocytes and decrease of ^{125}I incorporation and T4 and T3 secretion from human thyrocytes (34, 39, 40). TNF*α* plays an important role in the acute phase response and *in vitro* studies showed that it inhibits the TSH-induced cAMP response and thyroglobulin production and release in cultured thyrocytes (39, 41, 42).

Some evidence shows that pro-inflammatory cytokines may contribute to development of ESS also by affecting the expression and the activity levels of the deiodinases. In particular, during inflammation, competition for co-factors by cytokine-induced pathways such as nuclear factor-kappa B (NF-kB) are associated with less transcription of the D1 gene in the liver and with increase in D2 expression in the hypothalamus (43–45).

Although with the limitation of a small number of patients our data suggest that both adaptation and cytokines hyperproduction probably act together to development of ESS which entity, in our cohort, directly correlate with severity of clinical picture. Moreover, our data suggest that repeated dosages of fT3 levels may be useful to monitor the disease course.

In agreement with the role of ESS as useful and transient mechanism of adaptation during severe diseases, such as MIS-C, to reduce catabolic processes, in our cohort the levels of thyroid hormones spontaneously turned to normal during the first month from disease onset.

In our study, among the 36 subjects with ESS, only two had alterations of TSH or fT4 levels: these data differ from those reported in literature where the rate of ESS with an isolated reduction of fT3 was observed in only 65% of patients (4). In our patients diagnosis and treatment were started quite early after disease onset, therefore this could suggest a greater peripheral block of conversion of fT3 than of fT4 and a lack of central inhibition of TSH in our cohort.

In our study patients who needed admission to ICU presented lower levels of fT3 and moreover we showed a strong direct correlation between fT3 levels and severity of myocardial depression on admission. In fact we retrieved that suppression of fT3 was associated with EF < 45% and inversely correlated with BNP levels.

The relationship between ESS and heart dysfunction has been extensively investigated. Low serum fT3 concentrations are a negative prognostic factor in patients with congestive heart failure, raising a question whether thyroid hormones may play a role in acute cardiac injury (46, 47). In children undergoing cardiac surgery more severe ESS changes are associated with prolonged hospital stays and increased ICU and mechanical ventilation requirements (48, 49).

A possible explanation of this relationship is that in cardiomyocytes, deiodinase D3 expression is low under physiological conditions while its activity is upregulated in case of myocardial infarction (50, 51). Another possible contributing factor is hypoxia because increased level of peripheral D3 are induced by hypoxemia due to decreased tissue perfusion during illness (52, 53).

Our study has some limitation due to the small number of patients secondary to the low prevalence of MIS-C and the monocentric nature of the study. Despite the very different samples size the post-hoc power analysis was very good, thus confirming the statistical significance of our findings. Indeed, further studies on larger populations should be needed to confirm the data.

Conclusion

Our study showed that most of MIS-C patients has ESS, mainly characterized by low levels of fT3. In addiction, results suggest that ESS, particularly fT3 level, is an independent risk factor for the disease severity of MIS-C. In fact, patients with ESS and lower fT3 had stronger inflammatory responses, more severe cardiac involvement and higher risk of ICU requirement. These findings may be helpful in clinical practice because fT3 is processed by conventional laboratory in urgency, therefore it could be used as an additional data in prompt decision making in patients with MIS-C.

Data availability statement

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

Ethics statement

This study was approved by Ethical Committees of Padua University.

Author contributions

MF data collection and analysis and manuscript writing, AM data collection and manuscript revision, JG data interpretation and manuscript revision, FV data analysis and interpretation, FT data collection and manuscript revision, FZ data interpretation and manuscript revision, GM study design, data analysis and interpretation and manuscript writing. All authors contributed to the article and approved the submitted version.

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.

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Supplementary material

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Outcomes of MIS-C patients treated with anakinra: a retrospective multicenter national study

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Background: The treatment of multisystem inflammatory syndrome in children unresponsive to first-line therapies (IVIG and/or steroids) is challenging. The effectiveness of IL-1 receptor antagonist, anakinra, is debated.

Patients and methods: We conducted an anonymous retrospective multicenter study on MIS-C patients treated with anakinra in Italy from January 2020 to February 2021. Our study outcomes included the percentage of patients who required further therapeutic step-up, the percentage of patients who experienced fever resolution within 24 h and a reduction of CRP by half within 48 h, and the percentage of patients who developed Coronary Artery Anomalies (CAA) during follow-up.

Results: 35 cases of MIS-C were treated in 10 hospitals. Of these, 13 patients started anakinra while in the ICU, and 22 patients started anakinra in other wards. 25 patients (71.4%) were treated with corticosteroids at a starting dose 2–30 mg/Kg/day plus IVIG (2 g/Kg), 10 patients (28.6%) received only corticosteroids without IVIG. Anakinra was administered intravenously to all patients in Group A (mean dose 8 mg/Kg/day), and subcutaneously in Group B (mean dose 4 mg/Kg/day). Only two patients required further treatment step-up and no patients developed CAA after receiving anakinra. The most commonly observed side effect was an increase in ALT, occurring in 17.1% of patients.

Abbreviations:
MIS-C, multisystem inflammatory syndrome in children; ICU, intensive care unit; IVIG, intravenous immunoglobulin; CAA, coronary artery anomalies (CAA); AHA, American heart association; CRP, C-reactive protein; Pro-BNP, pro-brain natriuretic peptide; EF, ejection fraction.

Conclusions: In this retrospective cohort of severe MIS-C patients treated with anakinra we report favorable clinical outcomes with a low incidence of side effects. The simultaneous use of steroids ± IVIG in these patients hinders definitive conclusions regarding the need of IL-1 inhibition in MIS-C treatment.

KEYWORDS

MIS-C, multisystem inflammatory syndrome, pediatrics, COVID-19, SARS-CoV2

Introduction

MIS-C is a severe complication of SARS-CoV2 infection in children, it often results in high rates of ICU admission, mainly due to acute cardiac insufficiency. Although most patients do not develop sequelae, in the acute phase MIS-C can be a life-threatening disease, making timely and appropriate treatment critical (1). Currently, the primary treatment options for MIS-C are IVIG and steroids. Numerous studies have established that MIS-C results from a cytokine storm involving various cytokines (2, 3). Therefore, biologic drugs, particularly the IL-1 receptor antagonist (anakinra), have shown promise in treating MIS-C, as evidenced by small case series. However, data on the overall efficacy and safety of anakinra in MIS-C treatment is still lacking (4–9).

Methods

We conducted an anonymous retrospective multicenter study on patients who met the criteria for MIS-C and were treated with anakinra in Italy from January 4th, 2020 to February 28th, 2021. We included patients who met the WHO preliminary case definition (10) with recent biological evidence of SARS-CoV2 infection, either through serology or positive RT-PCR swab tests within six weeks prior to admission. The patients were divided into two groups: Group A included those who began anakinra treatment in the ICU, while Group B included patients treated in non-ICU pediatric wards. We collected clinical data at symptom onset, at the start of anakinra treatment, previous and concurrent treatments, along with reasons why anakinra was prescribed, dosage, and route of administration. The reasons for starting anakinra, according to the prescriber, included: worsening of cardiac dysfunction (defined as a reduction of EF to less than 50% or persistent hypotension requiring inotrope support), persistent clinical symptoms (persistence of fever with or without persistent mucocutaneous manifestations), or abnormal laboratory results (failure to reduce CRP or progressive increase of ferritin 24–36 h) despite treatment with steroid ± IVIG. We considered as outcomes the percentage of patients who required further therapeutic step-up, the percentage of patients with fever resolution within 24 h and CRP reduction by half within 48 h, and the percentage of patients with Coronary Artery Anomalies (CAA) during follow-up. We defined CAA based on the 2017 AHA Guidelines for Kawasaki Disease (11). The presence of CAA was screened at anakinra start, 2 weeks and 6–8 weeks after disease onset. In addition, we calculated the halving time of pro-BNP/BNP and CRP for each patient.

Results

Overall, 35 cases of MIS-C were treated in 10 hospitals throughout Italy and met the inclusion criteria, with 13 in Group A and 22 in Group B. **Table 1** displays baseline clinical and laboratory features, while **Table 2** provides information on first-line therapies and dosing, timing and reasons for anakinra initiation, anakinra dose, and method of administration. 25 patients were treated with corticosteroids at a starting dose 2–30 mg/Kg/day plus IVIG (2 g/Kg), 10 patients received only corticosteroids without IVIG. In Group A, cardiac function deterioration was the most common reason for starting anakinra (76.9%), while in Group B, anakinra was mostly started due to persistent clinical symptoms that were unresponsive to IVIG and/or steroids (54.5%). Of the 13 cases in Group A, 11 were admitted to ICU and initiated anakinra within 48 h of hospitalization. In all these patients anakinra was started less then 36 h after the start of steroid and/or IVIG. Anakinra was administered intravenously to all patients in Group A, with a mean dose of 8 mg/Kg/day. Meanwhile, the majority of patients in Group B (72.7%) received the drug via subcutaneous route, with a mean dose of 4 mg/Kg/day. **Table 3** outlines the outcomes and side effects experienced by these patients. In summary, only two patients required further treatment step-up: one patient in Group A needed an increase in methylprednisolone dose, while another patient in Group B required IVIG. No patients developed CAA after receiving anakinra. The most commonly observed side effect was an increase in ALT with 30.8% of Group A patients and 9.1% of Group B patients experiencing this effect. Only one patient in the study experienced an injection site reaction.

Discussion

MIS-C is a life-threatening late complication of SARS-COV2 infection in children. The treatment of MIS-C has been extensively debated over the past few years and is mainly based on the use of IVIG and/or glucocorticoids. According to most international recommendations, (12, 13) anakinra is suggested as a second-line therapy in patients resistant to conventional therapy. These recommendations are based mainly on individual case or small case series. A recent revision by Mastrolia et al. of these published cases found an efficacy of anakinra in 85/87 (97.7%) patients, nevertheless this result is burdened by publication bias and the outcome measures are extremely heterogenous (14). In this report, we describe the outcomes of a

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TABLE 1 Baseline clinical and laboratory features.

	Group A	Group B
N. of patients	13 (37.1%)	22 (62.9%)
Age at diagnosis (years)	12 (8–13)	8.5 (4–12)
Positive SARS-CoV2 swab in the six weeks previous to admission	84.6%	31.8%
IgG SARS-Cov2 positivity	100%	100%
Clinical presentation		
Days of fever	5 (4–6)	5 (4–6)
Conjunctivitis	53.8%	81.8%
Mucositis	23.1%	54.5%
Lymphadenopathy	23.1%	45.5%
Hands and feet erythema/oedema	7.7%	18.2%
Rash	46.2%	50.0%
Diarrhea	61.5%	50.0%
Abdominal pain	61.5%	86.4%
Hypotension[a]	61.5%	40.9%
Blood exams (at anakinra start)		
Platelets (/mmc3)	185 (107–189)	152 (89–187)
WBC (/mmc3)	10,780 (5,020–14,280)	10,935 (6,155–18,500)
Lymphocytes (/mmc3)	960 (660–980)	1,710 (760–1,970)
Neutrophil (/mmc3)	9,820 (4,280–13,310)	7,175 (4,370–17,020)
AST (UI/L)	40 (27–69)	31 (25–58)
ALT (UI/L)	33 (21–53)	40 (20–47)
Na (mmol/L)	133 (132–135)	137 (134–138)
Albumin (g/dl)	3.1 (2.7–3.4)	2.9 (2.4–3.1)
CRP (mg/L)	293 (212–305)	86 (17–153)
Procalcitonin (ng/ml)	11.3 (3.5–49.0)	1 (0.3–12.6)
Ferritin (ng/ml)	974 (419–4,006)	598 (447–1,366)
Cardio-pulmonary alterations (at anakinra start)		
Hypotension[a]	69.2%	36.4%
Need of inotrope support	69.2%	0%
Ejection fraction ≤50%	75.0%	23.8%
Pericardial effusion	44.4%	38.1%
BNP (ng/ml)	–	49 (7–123)
proBNP (ng/ml)	16,549 (3,247–20,163)	6,043 (1,405–10,502)
CAA	0%	9,1%
Pleural effusion	30.8%	4.5%
Need of O2 supplementation	61.5%	13.6%

All the continuous variables are expressed as median and IQR.
MV, mechanical ventilation; NIV, noninvasive ventilation; HFNC, high flow nasal cannula; NC, nasal cannula.
[a]Below 5° percentile, according to age, gender, and height adjusted charts (Schwandt et al. Am J Hypertens. 2015).

large multicenter cohort of MIS-C patients treated with anakinra. In both Italy and the US, anakinra is considered a second-line therapy for MIS-C (12, 13). Therefore, our retrospective cohort includes patients who either had very severe MIS-C at onset or were unresponsive to first-line therapy. Despite this selection bias, the majority of our patients achieved positive clinical and laboratory outcomes. Specifically, 85.7% of patients experienced fever remission within 24 h, while the median CRP and pro-BNP halving time was 2 days. Recently, Çağlayan et al. published a retrospective study of 82 MIS-C patients treated with anakinra. Of them, 89.1% were discharged without sequelae, but seven patients died (8.5%). In contrast, none of the patients in our study died. The lower mortality in our cohort may be due to several possible factors, such as a different ethnic background and a higher mean dose of anakinra (5.5 mg/Kg/day vs. 2.7 mg/Kg/day in Çağlayan et al.) (9). Our study confirms that anakinra may have a role as a second line treatment after glucocorticoids and/or IVIG. In Group B, only one patient (4.5%) required further therapeutic intervention after starting anakinra, none required admission to the ICU or vasopressor support. The majority experienced rapid fever resolution (77.3% at +24 h) and normalization of EF within an average of three days. It should be highlighted that 10 patients did not receive IVIG prior to or during anakinra treatment. Of these patients, 90% achieved fever remission within 24 h and halved their CRP levels within 48 h when treated with a combination of anakinra and intravenous methylprednisolone, only 1 patient required further therapeutic step-up with IVIG. This suggests that the anakinra and intravenous methylprednisolone combination may be effective even without IVIG. After the first revision of our manuscript, Chang et al. published a multicenter retrospective study analyzing the effect of anakinra therapy in the early phase of MIS-C treatment. The authors compared 121 MIS-C patients treated with anakinra plus IVIG and/or glucocorticoids to 389

TABLE 2 Reasons for anakinra start, anakinra dose and route of administration.

Reason for anakinra prescription		
Worsening of cardiac dysfunction[a]	76.9%	45.4%
Persistent clinical symptoms[a]	38.5%	54.5%
Persistent alteration of blood exams abnormalities[a]	46.2%	49.9%
Anakinra prescription		
Time between admission and anakinra start (days)	1 (1–2)	3 (1–7)
Iv administration	100%	27.3%
Sc administration	0%	72.7%
Mean dose (mg/kg/day)	8 (6.4–10)	4 (4–5.2)
Duration of anakinra treatment (days, with tapering)	35 (32–35)	27 (14–54)
First-line therapy		
Patients treated with IVIG (2 g/kg) without corticosteroids	0%	0%
Patients treated with IVIG (2 g/kg) + corticosteroids (2–30 mg/kg/day iv)	69.2%	72.7%
Patients treated with corticosteroids (2–30 mg/kg/day iv)	30.8%	27.3%

All the continuous variables are expressed as median and IQR.
[a]Multiple choices were possible.

TABLE 3 Outcomes and side effects.

	Group A	Group B
N. of patients	13 (37.1%)	22 (62.9%)
Outcomes		
Need of treatment step-up after anakinra	7.7%	4.5%
Defervescence of fever at 24 h	100%	77.3%
CRP halving at 48 h	76.9%	72.7%
CAA development after anakinra	0%	0%
CRP halving time (days)	2 (1–2)	2 (1–3)
BNP/proBNP halving time (days)	2.5 (2–3)	2 (2–4)
Ejection Fraction normalization time (days)	2 (2–3)	3 (2–9)
Need of ICU admission after anakinra	–	0%
ICU admission duration after anakinra	5 (4–11)	–
Time to inotrope support halving (days)	2 (1–3)	–
MIS-C relapse after anakinra suspension	0%	0%
Side effects		
ALT increase during treatment (>2× age range)	30.8%	9.1%
Injection site reaction	–	4.5%

All the continuous variables are expressed as median and IQR.

propensity-score matched MIS-C patients treated without anakinra, and found that the treatment with anakinra was not associated with significant differences in vasopressor requirement, ventricular dysfunction, or C-reactive protein reduction (15). The results of this recently published paper are partially comparable with ours, in fact Chang et al. only included patients who received anakinra on days 0 or 1 after diagnosis, which matches with only 11 patients in our cohort who were treated early with anakinra in the ICU setting. When comparing this similar subgroup of patients our outcomes were overall better than Chang's. For instance, the mean time to EF recovery was considerably shorter in our cohort (2 vs. 3 days). A possible explanation for this different outcome could be the higher mean dose of anakinra in our cohort (8 vs. 4 mg/Kg/day). It is worth mentioning that anti-IL1Ra autoantibodies have been described in small groups of children with MIS-C, and although their role in MIS-C pathogenesis is still debated, they can reduce free IL1-Ra, thereby possibly antagonizing anakinra and increasing the need for anakinra in MIS-C treatment (16). In accordance with this hypothesis, previous papers report the efficacy of high doses of anakinra in MIS-C (up to 10 mg/Kg/day) (5, 17). With regards to vasopressor support, the outcome considered in the aforementioned paper (% of patients needing vasopressor support at day 3) might not have been sensitive enough to detect the benefit of anakinra. In fact, in our experience, anakinra had a benefit in terms of vasopressor tapering (mean time for halving vasopressor dose was 48 h), but not in terms of early suspension. Finally, it should be highlighted that the incidence of CAA in MIS-C worldwide is between 10%–20%. In 2020, an Italian multicenter survey reported an incidence of 13.2% (12, 18). Interestingly, none of the patients in our cohort developed CAA after receiving anakinra, possibly indicating a protective role of IL-1 inhibition on coronary arteries during the acute phase of the disease, similar to that observed in KD (19).

This study has some limitations. Firstly, the data were collected retrospectively and there was no shared protocol between centers on when and how starting anakinra. The lack of a protocol led to a heterogeneity in timing and dosing between different hospitals. Additionally, the absence of a control group hinders the ability to draw a firm conclusion regarding the benefits of anakinra, particularly at disease onset when anti-IL1 is initiated shortly after steroid use.

In summary, in this retrospective cohort of patients treated with anakinra, we have observed favorable clinical outcomes with a low incidence of side effects, mainly transient ALT elevation. However, it is crucial to note that anakinra was administered in combination with other therapies, primarily steroids, which makes it difficult to draw definitive conclusions about the role of IL-1 inhibition in MIS-C treatment. Our data is not conclusive, especially in the ICU setting where anakinra was used simultaneously with IVIG and steroids.

Prospective randomized trials should be conducted to confirm our findings and determine the optimal timing and dose of anakinra for MIS-C treatment. Additionally, the development of a first-line therapies resistance score, similar to the Kobayashi score for IVIG resistance in Kawasaki Disease, might help identify which patients might benefit from prompt IL-1 inhibition.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Comitato etico interaziendale AOU Città della Salute e della Scienza di Torino—AO Mauriziano—ASL Città di Torino. Written informed consent to participate in this

study was provided by the participants' legal guardian/next of kin.

Author contributions

FL: conception and design of the work; analysis and interpretation of data; writing of the manuscript. CC: conception and design of the work; analysis and data collection; writing of the manuscript. MD: analysis and data collection; writing of the manuscript. NO: data collection and critical reviewing of the manuscript. MVM: data collection and critical reviewing of the manuscript. ALV: data collection and critical reviewing of the manuscript. VM: data collection and critical reviewing of the manuscript. MT: data collection and critical reviewing of the manuscript. AM: data collection and critical reviewing of the manuscript. MA: data collection and critical reviewing of the manuscript. GF: data collection and critical reviewing of the manuscript. MC: data collection and critical reviewing of the manuscript. AT: data collection and critical reviewing of the manuscript. RC: data collection and critical reviewing of the manuscript. GLM: data collection and critical reviewing of the manuscript. FLT: data collection and critical reviewing of the manuscript. AC: data collection and critical reviewing of the manuscript. GS: data collection and critical reviewing of the manuscript. AR: data collection and critical reviewing of the manuscript. DM: conception and design of the work; analysis and interpretation of data; critical reviewing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

GF is a consultant for SOBI, GS and MM received an unrestricted educational Grant from SOBI, for a project not related to MIS-C.

The remaining 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.

Publisher's note

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Demographic, clinical and laboratory differences between paediatric acute COVID-19 and PIMS-TS—results from a single centre study in the UK

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Background: Paediatric symptomatic SARS-CoV-2 infections associate with two presentations, acute COVID-19 and paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS). Phenotypic comparisons, and reports on predictive markers for disease courses are sparse and preliminary.

Methods: A chart review of COVID-19 and PIMS-TS patients (≤19 years) admitted to Alder Hey Children's NHS Foundation Trust, a tertiary centre in the North-West of England, was performed (02/2020–09/2022).

Results: A total of 161 symptomatic COVID-19 and 50 PIMS-TS patients were included. Peaks in admissions of patients with PIMS-TS occurred approximately 4 weeks after those for acute COVID-19. The incidence of in-patients with PIMS-TS reduced over time, and there were no admissions after February 2022. When compared to acute COVID-19, PIMS-TS patients were older (median: 10.3 years vs. 2.03 years; $p < 0.001$). There were no differences in gender distribution, but minority ethnicities were over-represented among PIMS-TS patients. Regional ethnic distribution was reflected among acute COVID-19 patients (66% vs. 84.5% White Caucasian, $p = 0.01$). Pre-existing comorbidities were more common among acute COVID-19 patients (54.7% vs. 8%, $p < 0.001$). PIMS-TS patients more commonly presented with abdominal symptoms (92% vs. 50.3%), neurological symptoms (28% vs. 10.6%) and skin rashes (72% vs. 16.8%), ($p \leq 0.01$) when compared with acute COVID-19, where respiratory symptoms were more common (51.6% vs. 32%, $p = 0.016$). PIMS-TS more frequently required intensive care admission (64% vs. 16.8%), and inotropic support (64% vs. 9.3%) (all $p < 0.05$). More deaths occurred among acute COVID-19 patients [0 vs. 7 (4.4%)], with 5/7 (71%) in the context of pre-existing comorbidities. When compared to acute COVID-19, PIMS-TS patients exhibited more lymphopenia and thrombocytopenia, a more pronounced acute phase reaction, and more hyponatraemia ($p < 0.05$). Partial least square discriminant analysis of routine laboratory parameters allowed (incomplete) separation of patients at diagnosis, and variable importance projection (VIP) scoring revealed elevated CRP and low platelets as the most discriminatory parameters.

Conclusion: Admissions for PIMS-TS reduced with increasing seroconversion rates in the region. Young age and pre-existing comorbidities associate with hospital admission for acute COVID-19. While PIMS-TS may present more acutely with increased need for intensive care, acute COVID-19 had an increased risk of mortality in this cohort.

KEYWORDS

acute COVID-19 children, MIS-C multisystem inflammatory syndrome in children, PIMS-TS, retrospective study, epidemiology, clinical feature

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible for the COVID-19 pandemic (1). Since December 2019, COVID-19 presented in waves that associated with various SARS-CoV-2 variants of concern (VoC) which emerged over time (1, 2). New variants were the result of hypermutation, predominantly affecting the spike region of the virus' RNA genome (1, 3). Because the spike protein is responsible for the infection of host cells, virus variants exhibit variable pathogenicity (3).

Notably, SARS-CoV-2 infections can manifest in a variety of clinical pictures. While most patients, across age groups, are asymptomatic or exhibit mild upper respiratory symptoms, others become severely unwell, particularly those with underlying comorbidities and/or the elderly (4–6). Severe COVID-19 can result in organ failure and death (7, 8). Mortality rates in the United Kingdom (UK) between 2020 and 2021 were 113.8 per 100,000 (9). Mortality was 62 times higher among patients aged >65 years (6).

When compared to adults, children and young people (CYP) infected with SARS-CoV-2 exhibit reduced disease severity and lower mortality, with infections typically being mild or asymptomatic (10). However, a subset of children develop severe acute COVID-19 requiring hospitalisation (11–13). Mortality associated with acute COVID-19 is low in children when compared to adults (estimated 1.83/million CYP in England) (14). Similar to adults, CYP presenting with severe COVID-19 are more likely to have comorbidities (11, 13, 15–17). Pre-existing conditions associated with severe COVID-19 in CYP include chronic lung disease, airway anomalies, neurodevelopmental disorders, cardiovascular disease, prematurity, diabetes mellitus, obesity (13, 15), immunosuppression, and sickle cell disease (16). However, published reports on underlying risk factors for severe acute COVID-19 and hospitalisation in children were descriptive, limited in numbers, and sometimes contradictory.

A subset of CYP present with a post-infectious hyperinflammatory syndrome termed Paediatric Multisystem Inflammatory Syndrome Temporally Associated with SARS-CoV-2 (PIMS-TS) or multisystem inflammatory syndrome in children (MIS-C) (18, 19). PIMS-TS typically presents between two and six weeks after the exposure to SARS-CoV-2 (20). It is characterised by persistent fever, elevated laboratory markers of inflammation, and evidence of single or multi-organ dysfunction (21, 22). PIMS-TS represents a disease spectrum, and mild cases may be missed. The full clinical picture of PIMS-TS frequently includes gastrointestinal and cardiovascular involvement (23–25). In a study in the Czech Republic reporting cases between November 2020 and March 2022, the incidence of PIMS-TS was estimated as 53/100,000 SARS-CoV-2 positive children (26).

This study aimed to compare incidences (over time), clinical and laboratory features of PIMS-TS and acute COVID-19 in CYP admitted to a tertiary paediatric hospital in the North-West of England.

2. Methods

2.1. Study design and participants

A retrospective chart review was undertaken in CYP (≤19 years) admitted to Alder Hey Children's NHS Foundation Trust, Liverpool, for acute COVID-19 or PIMS-TS between 10/02/2020 to 31/08/2022. The study was approved as a service evaluation by the local audit committee.

2.2. Case definitions and ascertainment

Several different diagnostic criteria exist to define PIMS-TS/MIS-C. For this study, the case definition of the Royal College of Paediatrics and Child Health (RCPCH) was used (19). Patients were classified by the centre's PIMS-TS multidisciplinary team (including infectious disease, rheumatology, general paediatric and cardiology specialists).

Acute COVID-19 cases were defined as CYP with an acute hospital admission who tested positive for SARS-CoV-2 by RT-PCR (real time polymerase chain reaction) AND the admission was due to COVID-19. Hospital acquired acute COVID-19 cases were also included if patients tested positive for SARS-CoV-2 AND developed symptoms suggestive of acute COVID-19 during their admission. All in-patients testing positive for SARS-CoV-2 (RT-PCR) between 10/02/2020 to 31/08/2022 were identified from the microbiology database. Cases were screened and case definitions for symptomatic acute COVID-19 were applied by PJ and MM to exclude patients with incidentally positive SARS-CoV-2 RT-PCR (e.g., elective admissions, surgical procedures, admissions for mental health issues). Unclear cases were reviewed by CH, CP, WW.

Electronic medical records of inpatients meeting the definition of PIMS-TS or acute COVID-19 were accessed to record demographic (age, sex, and ethnicity) and clinical information, including date of admission, comorbidities, clinical symptoms and signs, laboratory parameters within 24 h of admission and peak abnormal values (including white blood cells [WBC], neutrophils, lymphocytes, C-reactive protein [CRP], ferritin, triglycerides, platelets, INR, fibrinogen, D-dimer, haemoglobin [Hb], sodium, urea, creatinine, bilirubin, alanine transaminase [ALT], aspartate aminotransferase [AST], and treatment. Disease severity as estimated by recording duration of admission [days], admission to critical care, level of medical intervention required (e.g., fluid resuscitation, inotropic support, non-invasive or invasive ventilatory support), and mortality was recorded.

2.3. Statistical analysis

Statistical analyses were performed in Rstudio 2022.07.2 (27), utilising R version 4.2.2 and the "tidyverse" package (28). Quantitative variables were reported using median and interquartile range (IQR), as most variables followed a non-parametric distribution. Continuous variables were tested for normal distribution using Shapiro-Wilk test ($p > 0.05$); *t*-tests were used for comparisons between groups if following normal distribution; Mann–Whitney tests were used for variables not following normal distribution. Categorical variables were compared between groups using Chi-Square tests or Fisher's exact test. Statistical significance was determined as a *p*-value below or equal to 0.05 ($p \leq 0.05$). Bonferroni correction was utilised when comparing clinical characteristics to adjust for multiple comparisons. Patients with missing laboratory data were excluded in the univariate analysis. Laboratory parameters with ≥95% missing data in either group were not compared in univariate or multivariate analysis. A partial least squares-discriminant analysis (PLS-DA) model and a variable importance plot of laboratory tests, were conducted on Rstudio using the "mixOmics" (29) and "tidyverse" package (28). For analysis of admission blood tests, laboratory tests with the fewest missing datapoints (≤16% for either group) were selected, and missing values were imputed by using the mean of the variable for each group. For analysis of peak blood tests, no imputation was done due to a greater percentage of missing datapoints, and any patients with missing values were omitted.

We chose the PLS-DA model, a supervised multivariate statistical method, as a dimensionality reduction tool to produce latent variables, which are a linear combination of the laboratory parameters. 95% confidence ellipses were used to visually estimate whether groups can be separated based on the laboratory parameters. A variable importance plot (VIP) of laboratory parameters was used to display which laboratory variables most contributed to the PLS-DA model. The variable importance plot produces VIP scores for each independent variable in the PLS-DA model, and thus the importance of each variable in contributing to the separation in the two groups of the PLS-DA model. This approach was used to identify which laboratory variables best predict whether patients would belong to the COVID-19 or PIMS-TS group.

3. Results

3.1. Demographics and epidemiology

During the study period a total of 50 patients met the case definition for PIMS-TS. Over the same interval, 848 CYP (≤19 years) tested positive for SARS-CoV-2 by RT-PCR. Of these, 687 CYP (81%) were asymptomatic and/or incidental and were excluded from the analysis. This left 161 patients who were admitted to hospital due to acute COVID-19 or developed symptomatic disease during hospital admission. Two CYP had two separate acute COVID-19 infections and were included in the analysis twice.

PIMS-TS patients were significantly older (median: 10.3 [IQR: 5.65] vs. 2.03 [IQR: 10.6] years; $p < 0.001$) (**Table 1**). No differences were recorded in gender distribution. Notably, while in the PIMS-TS cohort ethnic minorities were over-represented, the acute COVID-19 cohort reflected the regional ethnic distribution (63.6% vs. 83.8% White Caucasians, $p < 0.01$), with the White Caucasian ethnic group representing 84% of the regional population (National Census, 2021) (30).

Pre-existing comorbidities were more common among acute COVID-19 patients (42.2% vs. 4%, $p < 0.001$), and 28% had more than one comorbidity. Most common pre-existing diseases

TABLE 1 Demographics of CYP admitted with PIMS-TS and acute COVID-19.

Parameter		PIMS-TS ($n = 50$)	Acute COVID-19 ($n = 161$)	*p*-value
Median age (Interquartile range, IQR)		10.3 (5.65)	2.03 (10.6)	<0.001
Male to female ratio		1:1	1:0.77	0.418
Ethnicity	White caucasian % (*n*)	63.6 (28/44)	83.8 (119/142)	0.004
	Other % (*n*)	36.4 (16/44)	16.2 (23/142)	
	Not stated % (*n*)	12.0 (6/50)	11.7 (19/162)	
Any comorbidity % (*n*)		4.0 (2/50)	42.2 (68/161)	<0.001
	Neonate (<28 days old) % (*n*)	0.0 (0/50)	6.2 (10/161)	
	Prematurity in infant (<1 year old) % (*n*)	0.0 (0/50)	9.9 (16/161)	
	Neurological & neurodevelopmental % (*n*)	0.0 (0/50)	9.3 (15/161)	
	Cardiovascular % (*n*)	0.0 (0/50)	15.5 (25/161)	
	Metabolic/endocrine % (*n*)	0.0 (0/50)	8.7 (14/161)	
	Respiratory % (*n*)	4.0 (2/50)	9.9 (16/161)	
	Primary immunodeficiency % (*n*)	0.0 (0/50)	1.2 (2/161)	
	Secondary immunodeficiency % (*n*)	0.0 (0/50)	9.9 (16/161)	
	Oncological % (*n*)	0.0 (0/50)	5.0 (8/161)	
	Renal disease % (*n*)	0.0 (0/50)	7.5 (12/161)	
	Liver disease % (*n*)	0.0 (0/50)	1.9 (3/161)	
	Trisomy 21% (*n*)	0.0 (0/50)	1.2 (2/161)	
	Other % (*n*)	0.0 (0/50)	9.9 (16/161)	
	Multiple comorbidities (more than one) % (*n*)	0.0 (0/50)	27.3 (44/161)	

included cardiovascular disease (15.5%), then prematurity in infant (<1 year old), respiratory and secondary immunodeficiency (all 9.9%) (**Table 1**, **Supplementary Table 1**). The two PIMS-TS patients with comorbidities had asthma.

As previously reported, hospital admissions for PIMS-TS lagged approximately 4 weeks behind those for acute COVID-19 (**Figure 1**) (20, 33). No cases of PIMS-TS were recorded from February 2022, which coincided with the predominance of the SARS-CoV-2 Omicron variant (31).

3.2. Clinical presentation and disease severity

As per case definition, all PIMS-TS patients exhibited fevers during admission which compared to 72% of acute COVID-19 patients ($p < 0.001$) (**Table 2**). PIMS-TS patients more frequently presented with gastrointestinal symptoms (all $p < 0.001$), neurological symptoms (28% vs. 10.6%, $p = 0.0023$) and/or headaches (52 vs. 12.4%, $p < 0.001$). Furthermore, PIMS-TS patients more commonly exhibited symptoms also associated with "historic" Kawasaki disease. Patients admitted with acute COVID-19 more frequently exhibited upper respiratory symptoms (51.6% vs. 32%, $p = 0.015$). A total of 27 (16.8%) acute COVID-19 patients were admitted with feeding concerns, while no PIMS-TS patients were ($p = 0.001$).

To assess disease severity, admission to critical care, cardiorespiratory supportive therapy and duration of hospital admissions were compared (**Table 3**). Patients with PIMS-TS more frequently required oxygen supplementation and inotropic support (64% vs. 9.3%, $p < 0.001$) but no significant differences were observed in escalating respiratory support via non-invasive (NIV) or invasive ventilation. A higher proportion of critical care admissions occurred in the PIMS-TS when compared to the acute COVID-19 cohort (64% vs. 16.8%, $p < 0.001$). No PIMS-TS patients required extracorporeal membrane oxygenation (ECMO) whereas 3 (1.9%) patients with acute COVID-19 did ($p = 0.3$). PIMS-TS patients had a longer median hospitalisation (8 vs. 3 days, $p < 0.001$). Seven patients admitted with acute COVID-19 died, while all PIMS-TS patients in this cohort recovered (Box 1).

3.3. Laboratory phenotype and predictive markers

PIMS-TS patients exhibited higher systemic markers of inflammation when compared to acute COVID-19 patients, including leucocytosis ($p = 0.01$), neutrophilia and elevated CRP ($p < 0.001$) (**Table 4**). While ferritin ($p = 0.01$), fibrinogen ($p < 0.001$) and D-dimers ($p = 0.028$) were higher in PIMS-TS patients, they were infrequently tested in acute COVID-19. PIMS-TS patients exhibited increased overall WBC but more

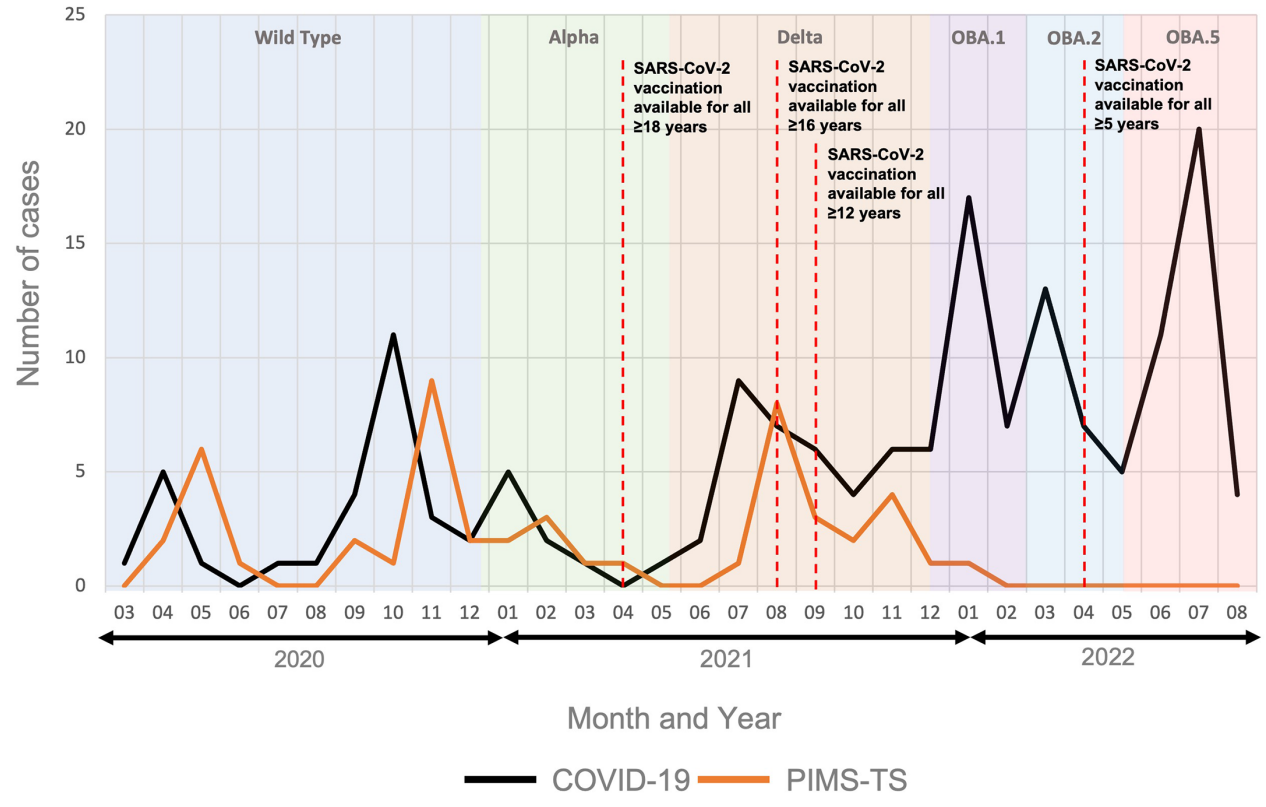

FIGURE 1
Hospital admissions for PIMS-TS and acute COVID-19. The figure includes data from 50 patients admitted for PIMS-TS and 161 acute COVID-19 patients. Predominant SARS-CoV-2 variants in the region are indicated over time. OBA.1: Omicron variant BA1; OBA.2: Omicron variant BA2; OBA.5: Omicron variant BA5 (31). Timelines of the SARS-CoV-2 vaccination roll out are indicated (32).

TABLE 2 Clinical phenotype of PIMS-TS and acute COVID-19 patients.

Clinical features		PIMS-TS ($n = 50$)	Acute COVID-19 ($n = 161$)	p-value
Gastrointestinal	Abdominal pain % (n)	82.0 (41/50)	19.3 (31/161)	<0.001*
	Diarrhoea % (n)	56.0 (28/50)	24.2 (39/161)	<0.001*
	Nausea/Vomiting % (n)	72.0 (36/50)	34.8 (56/161)	<0.001*
Respiratory	Upper respiratory tract symptoms or signs % (n)	32.0 (16/50)	51.6 (83/161)	0.015*
	Signs of increased work of breathing % (n)	24.0 (12/50)	31.7 (51/161)	0.3
Cardiovascular	Tachycardic on admission % (n)	62.0 (31/50)	52.2 (84/161)	0.22
Neurological	Confusion or neurological concern % (n)	28.0 (14/50)	10.6 (17/161)	0.0023*
	Headache % (n)	52.0 (26/50)	12.4 (20/161)	<0.001*
Bilateral non-purulent conjunctivitis % (n)		76.0 (38/50)	1.2 (2/161)	<0.001
Skin rash % (n)		72.0 (36/50)	16.8 (27/161)	<0.001
Mucous membrane changes % (n)		56.0 (28/50)	0.0 (0/161)	<0.001
Swelling to hands/feet % (n)		42.0 (21/50)	3.1 (5/161)	<0.001
Cervical lymphadenopathy % (n)		30.0 (15/50)	3.7 (6/161)	<0.001
Hepatosplenomegaly % (n)		8.0 (4/50)	5.6 (9/161)	0.53
Arthritis % (n)		6.0 (3/50)	0.0 (0/161)	0.002
Lethargy % (n)		6.0 (3/50)	12.4 (20/161)	0.203
Feeding concerns % (n)		0.0 (0/50)	16.8 (27/161)	0.0019
Fever on admission % (n)		84.0 (42/50)	59.6 (96/161)	0.0016
Fever at any point during admission % (n)		100.0 (50/50)	72.0 (116/161)	<0.001

*These comparisons remained significant after correcting using the Bonferroni method when making multiple comparisons within organ systems. p-values presented are before Bonferroni adjustment. No correction was applied to independent comparisons.

TABLE 3 Parameters of disease severity.

Parameters of disease severity		PIMS-TS ($n = 50$)	Acute COVID-19 ($n = 161$)	p-value
Respiratory	Oxygen requirement % (n)	48.0 (24/50)	29.8 (48/161)	0.017*
	Non-invasive ventilation % (n)	4.0 (2/50)	7.5 (12/161)	0.39
	Invasive ventilation % (n)	12.0 (6/50)	14.3 (23/161)	0.68
Cardiovascular	Hypotensive requiring fluid resuscitation % (n)	68.0 (34/50)	19.9 (32/161)	<0.001*
	Inotropic support % (n)	64.0 (32/50)	9.3 (15/161)	<0.001*
Median hospital admission time (days)		8.0	3.0	<0.001
Admission to critical care % (n)		64.0 (32/50)	16.8 (27/161)	<0.001
Extracorporeal membrane oxygenation (ECMO) requirement % (n)		0.0	1.9 (3/161)	0.33
Renal replacement therapy % (n)		2.0 (1/50)	2.5 (4/161)	0.84
Patient mortality % (n)		0.0	4.4 (7/161)	0.23

*These comparisons remained significant after correcting using the Bonferroni method when making multiple comparisons within organ systems. p-values presented are before Bonferroni adjustment. No correction was applied to independent comparisons.

frequent lymphocytopenia ($p < 0.001$) and thrombocytopenia ($p < 0.001$). Patients with PIMS-TS more frequently exhibited hyponatraemia ($p < 0.001$).

To investigate whether laboratory tests at admission differentiate PIMS-TS from acute COVID-19 patients, partial least-squares discriminant analysis (PLS-DA) was performed, including the following parameters: haemoglobin, platelets, white blood cells, neutrophils, lymphocyte counts, CRP, sodium, urea, and creatinine (**Figure 2**). While PLS-DA did not completely separate PIMS-TS from acute COVID-19 patients, variable importance projection (VIP) analysis showed that CRP elevation and reduced platelet numbers were more pronounced in PIMS-TS as compared to acute COVID-19 patients.

Considering peak changes of laboratory parameters, overall, PIMS-TS patients exhibited a more pronounced acute phase reaction when compared to COVID-19 infection (elevated WBC, neutrophils, CRP, fibrinogen) (**Supplementary Table 2**). However, features overlapped more than at admission and did not allow for clear separation between groups (**Supplementary Figure 1**). While overlapping significantly between groups, VIP analysis suggested elevated CRP and thrombopenia to be associated with PIMS-TS more than active COVID-19.

3.4. Treatment of PIMS-TS and COVID-19

Various drug therapies were used to treat PIMS-TS and acute COVID-19 (**Table 5**). The most common treatments in PIMS-TS included intravenous immunoglobulins (IVIG) (82%, 41/50) and methylprednisolone (76%, 38/50) Dexamethasone was prescribed in 14.9% (23/161) of acute COVID-19 patients. Furthermore, 16% (8/50) of PIMS-TS patients received biologic disease modifying anti-inflammatory therapy, which compared to 3.7% of acute COVID-19 patients. Treatments included the recombinant interleukin receptor antagonist anakinra (8%) and the IL-6 receptor antagonist tocilizumab (8%).

Remdesivir, a viral RNA polymerase inhibitor, was used in 10.6% of acute COVID-19 patients. Four patients (2.5%) received the recombinant anti-SARS-CoV-2 monoclonal antibody Sotrovimab and 1 patient (0.6%) received Ronapreve, a combination of anti-SARS-CoV-2 recombinant antibodies casirivimab and imdevimab. Almost all PIMS-TS patients (94%) received intravenous antimicrobial therapy, compared to 58.4% of acute COVID-19 patients ($p < 0.001$).

4. Discussion

This single centre retrospective study from a large tertiary children's hospital in the North-West of England, which provides healthcare to over 330,000 children and young people each year (34), reports varying proportions of COVID-19 and PIMS-TS patients over time. While PIMS-TS was the dominant cause of SAR-CoV-2 associated hospital admissions early in the pandemic, cases of admissions due to acute COVID-19 rose sharply from January 2022 with the emergence of the Omicron

BOX 1 Acute COVID-19 cases with fatal outcomes.

Most patients in this cohort who died due to acute COVID-19 were girls (5/7, 71%), with it appearing that SARS-CoV-2 Delta variant was the most common (5 Delta vs 2 Omicron variant). Only 1/7 had SARS-CoV-2 variant testing (confirming Delta variant), with the remaining 6/7 assumed their variant using the most prevalent VoC in the North-West at time of positive SARS-CoV-2 RT-PCR test. Patient age range varied from 7 days to 13 years old, and 5/7 (71%) had comorbidities:

- 2 premature neonates born at 32 and 35 weeks;
- 1 infant with cardiomyopathy;
- 4 school-aged children: two did not have pre-existing comorbidities (one died of cerebral haemorrhage, the other one with ARDS secondary to COVID-19, leading to necrotising pneumonia with empyema and consequently multi-organ failure), one with complex neuro-oncological disability (midline glioma) and one with metabolic (mitochondrial storage) disease.

Four children died due to respiratory deterioration, one due to cardiovascular, one with neurological deterioration and one with multi-organ failure. Six of the seven children received mechanical ventilation and cardiovascular support with inotropes. Two children received extracorporeal membrane oxygenation (ECMO).

TABLE 4 Laboratory test results at admission.

Blood tests	PIMS-TS Median (IQR)	PIMS-TS %, (*n*) *n* = 50[a]	Acute COVID-19 Median (IQR)	Acute COVID-19 %, (*n*) *n* = 161[a]	*p*-value
White blood cells (WBC) $\times 10^9$/L	9.75 (11.1)	100 (50/50)	7.94 (5.44)	85.1 (137/161)	0.01
Neutrophils $\times 10^9$/L	7.68 (9.18)	100 (50/50)	3.8 (5.1)	85.1 (137/161)	<0.001
Lymphocytes $\times 10^9$/L	0.795 (0.808)	100 (50/50)	2.07 (2.22)	84.5 (136/161)	<0.001
C-reactive protein (CRP) mg/L	206.0 (114)	100 (50/50)	4.0 (12)	84.5 (136/161)	<0.001
Ferritin ng/ml	781.0 (1,196)	96.0 (48/50)	324.0 (718)	9.3 (15/161)	0.01
Triglycerides mmol/L	1.8 (1.3)	86.0 (43/50)	0.96 (0.605)	6.8 (11/161)	0.002
Platelets $\times 10^9$/L	138.0 (86.5)	100 (50/50)	313.0 (190)	84.5 (136/161)	<0.001
INR	1.14 (0.153)	100 (50/50)	1.17 (0.265)	29.8 (48/161)	0.49
Fibrinogen g/L	5.0 (1.74)	100 (50/50)	2.4 (1.12)	18.6 (30/161)	<0.001
D-dimer ng/ml	3,610.0 (3,158)	96.0 (48/50)	1,758.0 (3,402)	9.3 (15/161)	0.028
Haemoglobin (Hb) g/L	116.0 (26.8)	100 (50/50)	116.0 (21)	85.1 (137/161)	0.72
Sodium mmol/L	133.0 (5)	100 (50/50)	138.0 (3)	87.0 (140/161)	<0.001
Urea mmol/L	4.85.0 (5.12)	100 (50/50)	3.4 (2.3)	87.0 (140/161)	<0.001
Creatinine mmol/L	51.0 (39.8)	100 (50/50)	32.0 (21.5)	87.0 (140/161)	<0.001
Bilirubin µmol/L	8.0 (6)	100 (50/50)	7.0 (9)	53.4 (86/161)	0.43
Alanine transaminase (ALT) iu/L	28.5 (34.5)	100 (50/50)	25.0 (29)	53.4 (86/161)	0.6
Aspartate aminotransferase (AST) iu/L	29.0 (31)	100 (50/50)	35.0 (27)	53.4 (86/161)	0.22

[a]Percentage and number of populations which underwent these laboratory tests on admission.

variant in the region. This coincided with a decline in hospitalisations for PIMS-TS. The incidence of admissions for SARS-CoV-2 associated disease at Alder Hey Hospital follows the pattern seen nationally with corresponding spikes in infection and admission at similar times (35). Several groups previously suggested decreasing numbers of PIMS-TS cases with the Delta (20, 36–38) and especially the Omicron variant (20, 36–40), despite a significant rise in the number of hospitalised acute COVID-19 cases. Notably, in contrast to some of these reports, we observed an increase in both acute COVID-19 and PIMS-TS cases with the Delta variant (41).

Two main theories exist on why PIMS-TS numbers declined with the Delta and/or the Omicron variant:

- One hypothesis suggests that increasing SARS-CoV-2 seroprevalence, through infection or vaccination, may have reduced the PIMS-TS risk (39, 42, 43). However, Sorg et al. suggested that vaccination did not significantly alter the PIMS-TS risk in Germany because all age groups, including those not eligible for vaccination, had falling rates of PIMS-TS (37). In the here presented study, it was difficult to capture whether children had been vaccinated due to a lack of documentation in medical records. However, a significant proportion of the study population were likely not vaccinated because, in the UK, vaccination programmes for 12- to 15-year-olds only began in September 2021, and for 5- to 11-year-olds in April 2022. Furthermore, vaccination rates in the North-West were the lowest in England (44). Notably, in the here reported cohort and others (23, 45, 46), children admitted for PIMS-TS were older when compared to those admitted for COVID-19 (median: 10.3 vs. 2.03 years). This may suggest increased risk for PIMS-TS in individuals that

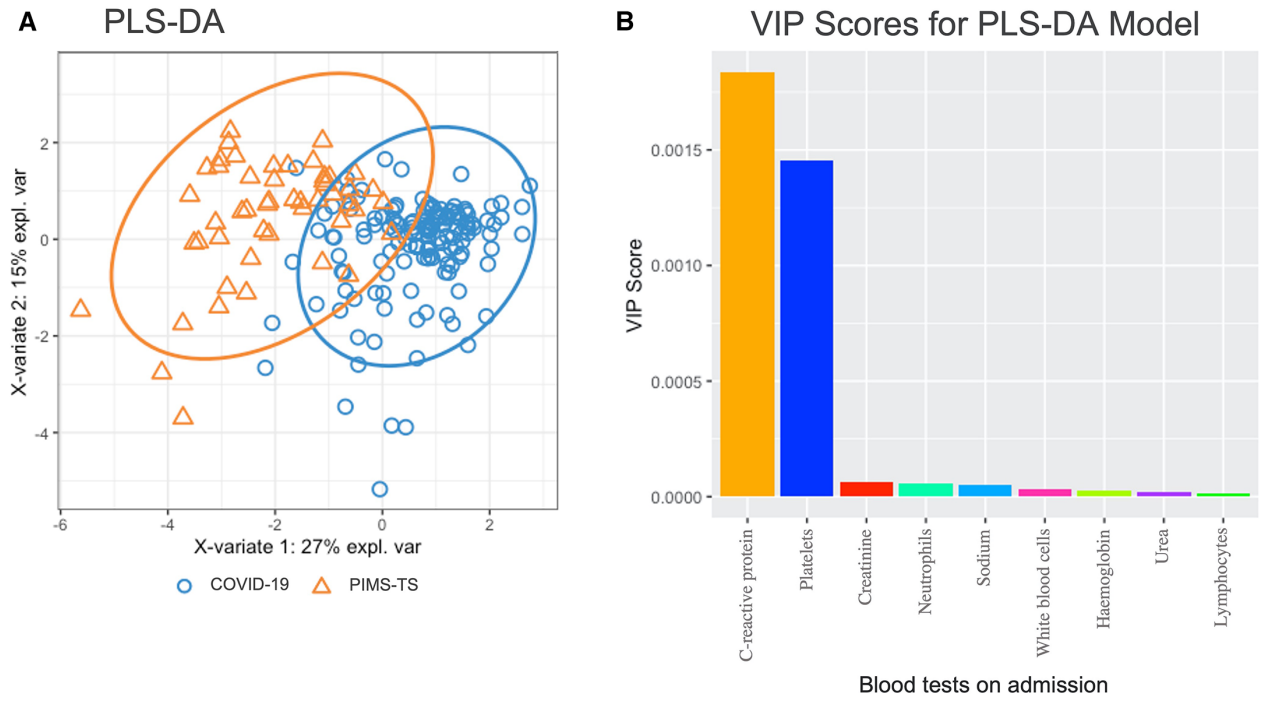

FIGURE 2
Partial least squares-discriminant analysis (PLS-DA) in PIMS-TS ($n = 50$) and acute COVID-19 ($n = 161$) patients at admission. Blood tests included were haemoglobin, platelets, white blood cells, neutrophils, lymphocytes, C-reactive protein, sodium, urea, creatinine. (**A**) PLS-DA analysis showing individual samples with the confidence ellipses of PIMS-TS and COVID-19. (**B**) VIP analysis showing variable importance plots of blood tests included in the PLS-DA model.

TABLE 5 Comparison of treatment in patients with PIMS-TS and acute COVID-19.

Treatment		PIMS-TS % (*n*)	Acute COVID-19 % (*n*)
Low dose aspirin (3–5 mg/kg/day)		96.0 (48/50)	0.6 (1/161)
High dose aspirin (30–50 mg/kg/day)		66.0 (33/50)	0.0 (0/161)
Intravenous immunoglobulin (IVIG)		82.0 (41/50)	1.9 (3/161)
Intravenous methylprednisolone		76.0 (38/50)	2.5 (4/161)
Tapering oral prednisolone following methylprednisolone		74.0 (37/50)	1.9 (3/161)
Dexamethasone		4.0 (2/50)	14.3 (23/161)
Biologics		16.0 (8/50)	3.7 (6/161)
	Anakinra	8.0 (4/50)	2.5 (4/161)
	Tocilizumab	8.0 (4/50)	1.2 (2/161)
Remdesivir		0.0 (0/50)	10.6 (17/161)
SARS-CoV-2 specific monoclonal antibodies		0.0 (0/50)	3.1 (5/161)
	Sotrovimab	0.0 (0/50)	2.5 (4/161)
	Ronapreve	0.0 (0/50)	0.6 (1/161)
Antimicrobials (intravenous)		94.0 (47/50)	58.4 (94/161)

first encounter SARS-CoV-2 in their school-age years as compared to younger children. Vaccination or infection early in life, which results in the acquisition of immune memory and an orchestrated innate and adaptive immune response against SARS-CoV-2, however, may prevent the development of PIMS-TS upon re-exposure later in life. Increasing and then constantly high case numbers among children in the region resulted in an estimated 93% of children having experienced SARS-CoV-2 infection by September 2022 (unpublished, UK Health Security Agency, UKHSA). This may explain the absence of admissions for PIMS-TS in the region after February 2022. Because vaccination may offer even better protection from PIMS-TS (including strong T cell responses) (47, 48), the observed disappearance of PIMS-TS over time may demonstrate the success of vaccination programmes, preventing hospitalisation and complications. Vaccination not only reduced COVID-19 severity (49), but may also have conferred protection against PIMS-TS (50–52). Therefore, a cornerstone of future public health planning in the face of disease outbreaks should focus on ensuring vaccine uptake remains high.

- A second theory claims that mutations in the virus' spike protein may affect infectivity, virulence, and disease outcomes (3, 53, 54). Reducing rates of PIMS-TS during the pre-vaccine Delta period in some studies suggests that changes to the SARS-CoV-2 virus may have been responsible. However, observations from the here presented study, namely increasing numbers of both COVID-19 and PIMS-TS during the "Delta variant wave", argue for increasing seroconversion through infection or vaccination having contributed to reduced numbers of PIMS-TS patient cases. Initially, during the first waves with the wild-type and alpha variants, children may have been less affected because of reduced travel activity, shielding, school closures in the regions and reduced infectivity and virulence within the age group (55–57). The proportion of PIMS-TS (as compared to COVID-19) were relatively high throughout the "Wild-type", "Alpha" and "Delta waves". Across the UK, approximately 40%

of 5- to 11-year-old and 82% of 12- to 18-year-old children and young people had detectable SARS-CoV-2 antibodies by December 2021, the end of the "Delta wave" (58). In adults, at a similar time point, >95% tested positive for SARS-CoV-2 antibodies (59). Of the 82.4% of 12- to 18-year-olds who tested positive for SARS-CoV-2 antibodies, less than half (43.1%) had received at least one COVID-19 vaccine dose (58).

Findings from this study support the first hypothesis, however it is likely, in our view that both theories have a part to play. At later stages, increased infectivity and virulence may have contributed to both increasing numbers of hospital admissions for acute COVID-19 and reduced proportions and absolute numbers of PIMS-TS patients.

This study confirms previous reports on PIMS-TS disproportionately affecting minority ethnic groups (23, 45, 60, 61). Notably, this contrasted with acute COVID-19 which reflected the regional composition of ethnic groups (62). While the exact reason for the predominance of ethnic minorities in PIMS-TS remain unknown, it suggests the involvement of genetic factors.

As mentioned above, children admitted for PIMS-TS were older children when compared to acute COVID-19, which is in line with previous reports (45, 46). However, young age also represents a possible risk factor for severe COVID-19 in children. Indeed, 39.8% (64/161) of patients admitted for COVID-19 in this study were younger than 1 year. This study also highlighted that children <1 year of age are at particular risk of becoming hospitalised with severe acute COVID-19. Notably, children >6 months have only recently become eligible for vaccination, but only if they are of an at-risk group (63). Given the risk profile, universal access to SARS-CoV-2 vaccines for infants, possibly through its incorporation into the childhood vaccination schedule, may prevent hospitalisation and even deaths in future wave outbreaks. Currently, the COVID-19 vaccine is not licenced for neonates, leaving this group highly vulnerable to severe acute infection. Thus, pregnant women should not only be encouraged to receive vaccines due to the increased risk of developing severe disease themselves, but also to allow effective maternal antibody transfer that may potentially protect infants in the crucial first 6 months of life (64, 65). As an alternative, in young children and infants who have not received vaccination, the use of neutralising monoclonal antibody products may incur survival benefit during severe acute COVID-19 disease (66), but more robust safety and efficacy data is required for its routine use.

Another factor associated with admission for COVID-19 is the presence of pre-existing comorbidities. While, in this study and others, only a few PIMS-TS patients (here 4%) had reported comorbidities (17, 23, 67, 68), 42.2% of COVID-19 patients had underlying disease (15, 69). Admissions for acute COVID-19 associate with a history of premature birth (13, 45), young age (<12 months) at infection (45, 61, 69, 70), type 1 diabetes (70), neurologic disorders (13, 45, 69, 70), cardiac disease (13, 45, 69, 70), respiratory disease (13, 45, 69), gastro-intestinal disease (45), malignancy (69), immunosuppression (16), chromosomal disorders including trisomy 21 (69), chronic kidney disease (69) and obesity (61). This suggests that the presence of comorbidities should be another key factor informing decisions on who to target in vaccination programmes and with therapies for non-hospitalised acute COVID-19 infections.

Patients admitted to hospital with PIMS-TS exhibited acute and severe disease but responded to anti-inflammatory and supportive treatment, which was in agreement with previous reports from us and others (45, 46, 61). When compared to COVID-19 patients, PIMS-TS patients had a greater length of admission (46), and an increased likelihood of critical care admission (45, 46). As with other studies, PIMS-TS associated with more gastrointestinal and neurological manifestations as well as mucocutaneous features (23, 45, 46, 60, 61, 71). Patients admitted for COVID-19 were, overall, less acutely unwell at admission. Nonetheless, 7/161 (4.3%) COVID-19 patients did not survive. In line with previous reports, and similar to the adult patient population, mortality in acute COVID-19 was associated with co-morbidities (15, 72). These observations are of relevance when, e.g., considering pre-hospitalisation treatment in high-risk patients (below).

At admission, the differentiation between acute COVID-19 and PIMS-TS can be challenging. Notably, while usually acute infection with SARS-CoV-2 precedes the onset of PIMS-TS by several weeks, some patients with PIMS-TS can remain PCR positive. This may be associated with persistent primary infection (73), or be the result of re-infection. In line with its hyper-inflammatory nature and previously reported features of "cytokine storm" (25, 74), PIMS-TS patients displayed markedly elevated markers of inflammation (white cell counts, neutrophils, C-reactive protein) (23, 45, 46, 60, 61, 71, 75). When compared to COVID-19, PIMS-TS associated with prominent lymphopenia and thrombocytopenia (23, 45, 46, 60, 61, 71, 75). However, severe COVID-19 may also present features of cytokine storm, such as pro-inflammatory cytokines and acute phase reactants, lymphopenia, and coagulopathy (76). Applying PLS-DA and VIP scoring, we identified CRP elevation and reduced platelet numbers as laboratory parameters with the highest contribution to differentiating PIMS-TS from acute COVID-19. Hyponatraemia, which was more common among PIMS-TS patients (46), may, as in "historic" Kawasaki disease, be explained by inappropriate antidiuretic hormone release (77). While these phenotype-associated features may aid in discriminating PIMS-TS from COVID-19, they are overlapping and require validation in larger independent patient cohorts, including further differential diagnoses such as other viral infections and bacterial sepsis (23, 45, 46, 60, 61, 71, 75).

In CYP, the evidence for treatment choices and associated risk is less robust when compared to the adult COVID-19 patient cohort (78, 79). At Alder Hey, treatment decisions were made by a multi-disciplinary team (including members from the infectious disease, rheumatology, general paediatrics, cardiology, and respiratory departments), which aided with balancing the relative effectiveness and safety of therapeutic options with changing virus variants over time. Treatment of acute COVID-19 with glucocorticosteroids was recommended from September 2020 in the UK, and was the mainstay of treatment for children requiring oxygen therapy (78). Remdesivir was licensed in July 2020, tocilizumab in December 2021 (78). Thus, these agents were only used in a minority of COVID-19 patients included here. For pre-

hospitalisation treatments to prevent severe COVID-19, Ronapreve (August 2021) and Sotrovimab (December 2021) were available for CYP aged 12 years old and older weighing at least 40 kg (78). Among PIMS-TS patients in this cohort, 60% received IVIG and intravenous glucocorticosteroids, and 4% received tocilizumab or anakinra, to which they had been randomised through the RECOVERY trial (80).

While adding to the understanding of phenotypical, laboratory and prognostic differences between PIMS-TS and COVID-19, this study has limitations. In this single-centre retrospective study, virus variant data was not available for the majority of patients and had to be estimated based on regional surveillance data over time. Medical records did not always contain ethnicity data, SARS-CoV-2 vaccination status or body mass index. Thus, we were unable to determine whether for e.g., obesity was a risk factor. Laboratory tests were more frequently performed in PIMS-TS patients when compared to acute COVID-19 patients, and patients with milder acute COVID-19 were less likely to undergo laboratory testing. Only complete cases were compared in univariate analysis of laboratory tests, and multi-variate analysis of peak laboratory tests, possibly allowing bias.

5. Conclusions

Both alterations to the SARS-CoV-2 spike protein and increasing seroconversion likely contributed to decreasing admissions for PIMS-TS over time. Especially in times with low PIMS-TS case numbers, its diagnosis and differentiation from acute COVID-19 can be challenging. Clinical features, including mucocutaneous and enteric symptoms, thrombocytopenia, and a significantly elevated CRP associate with PIMS-TS, which may aid diagnosis, and rapid commencement of effective therapy. Patients with PIMS-TS can be acutely ill but usually respond to anti-inflammatory and supportive measures. While a smaller proportion of paediatric patients with acute COVID-19 require intensive care admission, 4.3% did not survive. Severe disease and mortality in COVID-19 are associated with young age (infancy) and pre-existing health conditions, including cardiovascular, mitochondrial, and neurological disease. Thus, vaccine programmes should include young age groups and especially target children with pre-existing diseases.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

PJ and MM collected data. WW, CP and CH provided guidance on unclear cases of inclusion and exclusion. PJ, CP and CH wrote the manuscript. SL provided advice on statistics. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1219654/full#supplementary-material

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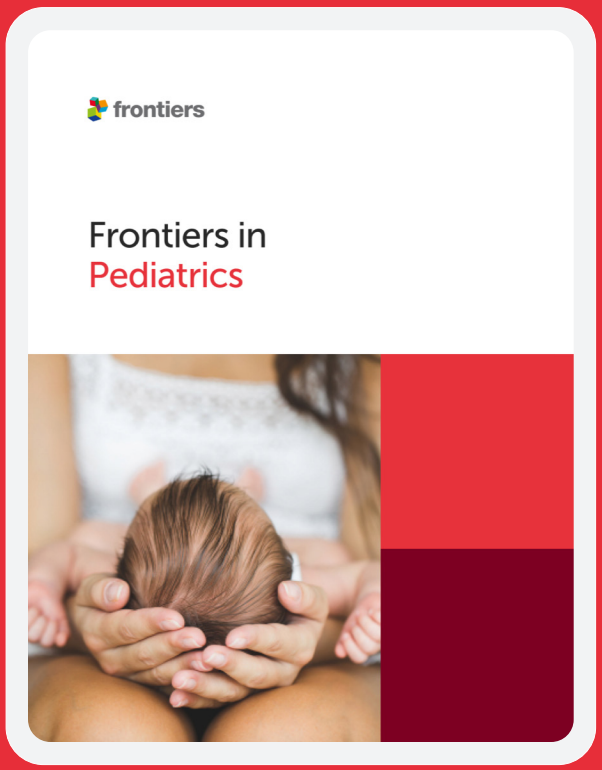

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