Multisystem inflammatory syndrome in children related to COVID-19: What we have learned so far on this new pediatric inflammatory syndrome

Grazioli, Serge

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

Clinical Medicine Section

Department of Pediatrics, Gynecology and Obstetrics

Multisystem inflammatory syndrome in children related to COVID-19: What we have learned so far on this new pediatric inflammatory syndrome

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Serge GRAZIOLI

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Abbreviations

ACE2: angiotensin-converting enzyme 2

ARDS: acute respiratory distress syndrome

CCL2: chemokine ligand 2

CDC: center for disease control and prevention

cDC: conventional dendritic cells

CMR: cardiac magnetic resonance

CNS: central nervous system

COVID-19: coronavirus disease 2019

CRP: C-reactive protein

CSF: cerebrospinal fluid

CT: computer tomography

CXCL9: C-X-C motif chemokine ligand 9

ECMO: extracorporeal membrane oxygenation

EEG: electroencephalogram

ESR: erythrocyte sedimentation rate

GI: gastrointestinal

KD: Kawasaki disease

IL-1: interleukin-1

IFN-γ: interferon gamma

IVIG: intravenous immunoglobulin

LV: left ventricular

MAS: macrophage activating syndrome

MERS-CoV: Middle East respiratory syndrome CoV

MCP-1: monocyte chemoattractant protein 1

MIS-C: multisystem inflammatory syndrome in children

MRI: magnetic resonance imaging

NK: natural killer

NM: nanometer

NT-proBNP: N-terminal-pro hormone B-type natriuretic peptide

pCD: plasmacytoid dendritic cells

PCR: polymerase chain reaction

PIMS-TS: pediatric inflammatory multisystem syndrome temporally associated with COVID-19

RCPH: Royal College of Pediatrics and Child Health

RESLES: reversible splenial lesions syndrome

ROTEM : rotational thromboelastometry

SARS-CoV-2: severe acute respiratory syndrome coronavirus type 2

STIR: short tau inversion recovery

TCR: T-cell receptor

TMPRSS2: transmembrane serine protease 2

TNF-α: tumor necrosis factor alpha

TSS: toxic shock syndrome

WHO: World Health Organization

Abstract

Since the beginning of COVID-19 pandemic, a new post-infectious inflammatory manifestation of SARS-CoV-2 affecting children has been described. It was defined as Pediatric Inflammatory Multisystem Syndrome temporally associated with COVID-19 (PIMS/PIMS-TS) by the Royal College of Pediatrics and Child Health, or as Multisystem Inflammatory Syndrome in Children (MIS-C) by the World Health Organization and the Center for Disease Control and Prevention. MIS-C represents a late manifestation of SARS-CoV-2 infection affecting predominantly previously healthy school-age children presenting with moderate to severe multi-organ dysfunction associated with an exaggerated proinflammatory response and presence of elevated SARS-CoV-2 immunoglobulin G antibodies. Although MIS-C shares similarities with Kawasaki Disease (KD), children with MIS-C tend to be older and present with a more severe proinflammatory response and myocardial injury than children with KD.

The pathophysiology of MIS-C remains largely unknown, and the current management of MIS-C is extrapolated from KD and focuses on restoring immune homeostasis with intravenous immunoglobulin, corticosteroids, and biologics as well as on the prevention of vascular complications with antiplatelet therapy and anticoagulation. Recent immunoprofile studies on MIS-C patients discovered the presence of a superantigenic-like motif on SARS-CoV-2 spike glycoprotein and an autoimmune signature targeting the various organs involved in MIS-C.

This review provides an extensive update on the state of knowledge of this new post-infectious hyperinflammatory syndrome affecting children. This topic is important for two reasons. Firstly, SARS-CoV-2 continues to infect children worldwide who may therefore still be at risk of developing MIS-C. The additional knowledge brought by this review to the clinicians may help them in the care of MIS-C patients. Secondly, a better understanding of the immunopathogenesis of MIS-C and how its immunopathological signature is distinct from acute SARS-CoV-2 infection or KD will not only be useful to guide optimal treatment in MIS-C but may also help in the management of similar hyperinflammatory conditions affecting children such as KD or haemophagocytic lymphohistiocytosis.

Introduction

Covid-19 pandemic

Coronaviruses are enveloped positive-sense single-stranded RNA viruses belonging to the Coronaviridae family (1) affecting both humans and animals and causing respiratory disease. Current taxonomy divides the subfamily *Coronavirinae* into four genera: Alphacornonavirus, Betacoronavirus, Gamacoronavirus and Deltacoronavirus. The Betacoronavirus genus includes the Severe Acute Respiratory Syndrome CoV (SARS-CoV) and Middle East Respiratory Syndrome CoV (MERS-CoV) responsible for SARS and MERS outbreaks that emerged in China between 2002-2003 and in the Middle East in 2012, and spread worldwide. In December 2019, an outbreak of patients presenting with an acute community-acquired atypical pneumonia of unknow etiology was reported in Wuhan, the capital of Hubei province in central China. In January 2020 the full draft genome sequence of the potential causative organism was released and identified as a new coronavirus belonging to the Betacoronavirus, named Severe Acute Respiratory Syndrome CoV-2 (SARS-CoV-2)(2, 3). Initially linked to a seafood and wet animal wholesale market in Wuhan, cases of patients diagnosed with this novel coronavirus disease, also known as Coronavirus Disease 2019 (COVID-19) started to spread very quickly, initially throughout China, then to neighboring countries, and finally worldwide. On March 2020, with more than 118’000 cases in 114 countries and more than 4000 death, the World Health Organization (WHO) declared COVID-19 caused by SARS-CoV-2 a new pandemic (4). At the time of writing, 6.6 million COVID-19 deaths have been reported by WHO and over 644 million cumulative cases globally since the start of the pandemic.

SARS-CoV-2 characteristics

SARS-CoV-2 has double-layered lipid envelope containing a non-segmented, single-stranded, positive-sense RNA genome of ~ 32 kilobases in size with a diameter of 50-200 nm. As a Betacoronavirus, SARS-CoV-2 shares 79% genome sequence identity with SARS-Co-V and 50% with MERS-CoV. All three coronavirus genomes encode for conserved structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The clublike spike projections of the S protein on the surface give the typical crown-like

appearance under the electronic microscope of these viruses and are responsible for their name after the Latin word corona, meaning crown or halo. The S protein play a significant role in viral attachment and host cell entry via the binding to angiotensin-converting enzyme 2 (ACE2) receptors (5). It has been shown that RNA viruses, such as the coronavirus, influenza virus and human immunodeficiency virus (HIV), present extremely high mutation rates as a result of their replication mechanism and the lack of viral RNA polymerase proofreading activity (6). Interestingly, the spike protein, which is the main target of the host immune system and a major point of interaction between the virus and the human immune system has been reported to undergo rapid molecular evolution and selection pressure during evolution (7). Since the first full characterization of the SARS-CoV-2 in Wuhan, numerous changes of amino acid on spike protein were described (8, 9) resulting in the emergence of several new coronavirus variants with differences in transmissibility and virulence.

Pathogenesis of SARS-CoV-2 infection

The primary route of entry of SARS-CoV-2 is through the upper respiratory tract, where it starts to replicate and then migrates down the airways and enters alveolar epithelial cells in the lungs, where the rapid replication of SARS-CoV-2 triggers a strong immune response. Exposed patients can be asymptomatic or develop mild to moderate symptoms with some patients suffering from severe disease that requires oxygen supplementation and sometimes intensive care support with mechanical ventilation (10). Autopsy reports showed bilateral diffuse alveolar damage together with hyaline membrane formation and pulmonary microemboli (11). Older patients and patients with serious pre-existing diseases are at greater risk of developing acute respiratory distress syndrome and death (12-14). Although COVID-19 presents mainly as a respiratory disease, SARS-CoV-2 infection causes extrapulmonary manifestations, in part as a result of the ubiquitous presence of ACE2 receptors in various tissues including the endothelium, gastrointestinal and renal tissues. Endothelium inflammation with the presence of viral particles identified by electron microscopy is another unique characteristic of SARS-COV-2 infection. Endotheliitis and soluble endothelial makers such as angiopoeiting-2 levels are positively correlated with severity of COVID-19 (15, 16) and associated with increased risk for thromboembolism and multisystem involvement in COVID-19 patients (17, 18). Patients with COVID-19 may also

present with several neurological manifestations such as SARS-CoV-2 associated meningitis, encephalitis, myelitis, acute disseminated encephalomyelitis, Guillain-Barré syndrome, stroke and taste and olfactory disturbances (19). It has been shown that SARS-CoV-2 can affect the nervous system in different ways with direct and indirect neuropathological effects. Central and peripheral nervous system damage could be the result of direct viral invasion via hematogenous or olfactory route.(20, 21). The viral infection can also lead to an immune-mediated central nervous system damage. Recent autopsy reports suggest that neurological manifestation in COVID-19 patients is principally mediated by microvascular and inflammatory mechanisms caused by viral induced systemic proinflammatory responses and endotheliitis rather than a direct viral cytopathic effect on neurons (20, 22-24). In a subgroup of COVID-19 patients, the infection is accompanied by an aggressive inflammatory response with the release of a large amount of proinflammatory cytokines including Tumor Necrosis Factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) in an event known as “cytokine storm”. This hyper-immune proinflammatory response causes multisystem manifestations leading to multiorgan failure, acute respiratory distress syndrome (ARDS), and unfavorable prognosis of severe COVID-19 (25-31).

COVID-19 in children

Since the beginning of the pandemic of COVID-19, numerous studies have shown that children and adolescents are susceptible to SARS-CoV-2 infection (32-34) without significant difference in age distribution among children (35). Unlike adults, children with COVID-19 very often present with nonspecific symptoms mimicking other pediatric diseases. A recent meta-analysis reported that fever and cough were present in respectively 51% and 41% of COVID-19 pediatric patients (35) which is lower than what is reported in adult COVID-19 patients, with fever and cough reported in respectively 78-92.8% and 57-63.4% (36-38). In addition of fever and respiratory symptoms, gastrointestinal symptoms are also very common in children with COVID-19 (39-41). While most children infected with SARS-CoV-2 are asymptomatic or present mild or moderate symptoms, approximately 6-7 % became severely or critically ill (35, 42). The risk for critical illness after SARS-CoV-2 infection was particularly higher in children under the age of one year or in children with comorbidities, when compared to healthy older children (35).

Multisystem Inflammatory Syndrome in Children

In the spring 2020 during the peak of the first wave of the COVID-19 pandemic, there were increasing reports of cluster of children in Europe and America presenting with hyperinflammatory shock and features resembling Kawasaki disease (KD) and Toxic Shock Syndrome (TSS) occurring in the weeks after SARS-CoV-2 infection or exposure (43-47). This new pediatric inflammatory syndrome was named multisystem inflammatory syndrome in children (MIS-C) by the US Centers for Disease Control and the WHO (48, 49), or Pediatric Inflammatory Multisystem Syndrome temporally associated with SARS-CoV-2 (PIMS-TS) by the UK Royal College of Paediatrics and Child Health (50). The definitions are summarized in Table 1 and require presence of fever, single or multi system involvement, inflammation, SARS-CoV-2 infection, and exclusion of other diagnoses. The exact incidence of MIS-C remains uncertain and its physiopathology, although actively investigated, remains unclear. Furthermore, although a majority of children diagnosed with MIS-C responded well to immunomodulatory treatments, the long term effect of this new inflammatory syndrome on these children remains unknown.

The purpose of this work is to provide a comprehensive overview of the current knowledge on this new inflammatory syndrome affecting children with a special focus on clinical presentation, immunopathogenesis, treatment and mid- and long-term outcomes.

Table 1. Case definitions of multisystem inflammatory syndrome in children

	Royal College of Pediatrics and Child Health (United Kingdom)	**CDC (United States)**	**WHO**
Name	Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS)	Multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19	Multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19
Age	Child	<21 years	0-19 years
Fever	Persistent fever > 38.5°C	Fever > 38°C for ≥ 24h	Fever for ≥ 3 days
Clinical features	Single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurologic disorder) with additional features	Severe illness requiring hospitalization with Multisystem involvement ≥ 2 organ system involved, cardiovascular, respiratory, renal neurologic, hematologic, gastrointestinal, dermatologic)	At least two of the following: (1) Rash, bilateral non-purulent conjunctivitis, or muco-cutaneous inflammation signs. (2) Hypotension or shock. (3) Cardiac dysfunction, pericarditis, valvulitis or coronary abnormalities. (4) Coagulopathy. (5) Acute gastrointestinal symptoms.
Covid-19 infection	SARS-CoV-2 RT-PCR positive or negative	Positive SARS-CoV-2 RT-PCR, or positive serology, or positive antigen test, or COVID-19 exposure within the 4 weeks before onset of symptoms	Positive SARS-CoV-2 RT-PCR, or positive serology, or positive antigen test, or COVID-19 exposure
Exclusion	Any other microbial cause	No alternative plausible diagnosis	No other obvious microbial cause of inflammation

CDC = Centers for Disease Control and Prevention; COVID-19 = coronavirus disease; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; RT-PCR = reverse transcription polymerase chain reaction; WHO = World Health Organization

Method

A narrative approach was chosen for this literature review, aiming at providing data on the clinical presentation, immunopathogenesis and outcomes of children presenting with MIS-C. References for this narrative literature review were identified through searches of PubMed and Google Scholar from 2019 until April 2022, using search terms relevant for each subsection, such as:
(coronavirus disease 19 OR COVID-19) and (children OR inflammation OR complications OR immune response OR treatment OR immunomodulation); (multisystem inflammation syndrome in children OR MIS-C OR Pediatric Inflammatory Multisystem Syndrome temporally associated with COVID-19 OR PIMS-TS) AND (diagnosis OR symptoms OR cardiovascular OR neurological OR gastrointestinal OR shock OR Kawasaki disease OR inflammation OR cytokines OR immune response OR treatment OR immunology OR immunomodulation OR cytokine storm OR coagulopathy OR complications OR outcome) as appropriate.
References were additionally identified though searches of the reference lists of already included articles. Only papers published in English were reviewed and the final reference list was based on the papers identified and deemed to be the most cited, high-quality, accessible, and relevant to the current narrative literature review on MIS-C.

Discussion

1.0 MIS-C epidemiology

The exact incidence of MIS-C is uncertain and may vary by patient characteristics such as age, sex, race, and geographic location. Dufort et al reported an overall incidence of MIS-C in New York state of 2 cases per 100'000 persons younger than 21 years during March 1 to June 30, 2020 (51). Regarding the incidence of MIS-C per infected children, it has been reported that MIS-C occurred in < 1% of children infected with SARS-CoV-2 with an estimated incidence of 31.6 out of 100'000 infected children(52). Unlike severe acute COVID-19 illness in children, which affects more often children with underling healthy problems, MIS-C is affecting typically healthy children with a median age between 8 to 11 years old (range 1 to 20 years old) and slightly higher proportion of boys (57%). The most frequent reported comorbidities in MIS-C patients were asthma and obesity with children from some ethnic groups such as the African-Americans and Hispanic communities being overrepresented (45, 46, 51, 53). MIS-C cases were typically observed following the peak incidence of COVID-19 in the general population and usually 2-6 weeks after SARS-CoV-2 infection (51, 53). By June 2022, a total of approximately 232 MIS-C cases were reported in Switzerland and the distribution of the cases in Geneva overtime is illustrated in Figure 1. The graph shows 3 peaks for MIS-C in April 2020, in November-December 2020, and in late December 2021-January 2022. Interestingly, the first 2 peaks followed the COVID-19 children and adults peaks by approximately 2-5 weeks. We also noticed that after the second MIS-C peak, there were continuous MIS-C cases from January until end of April 2021. The timing of the MIS-C cases related to the COVID-19 pandemic in Geneva is similar to what has been reported worldwide.

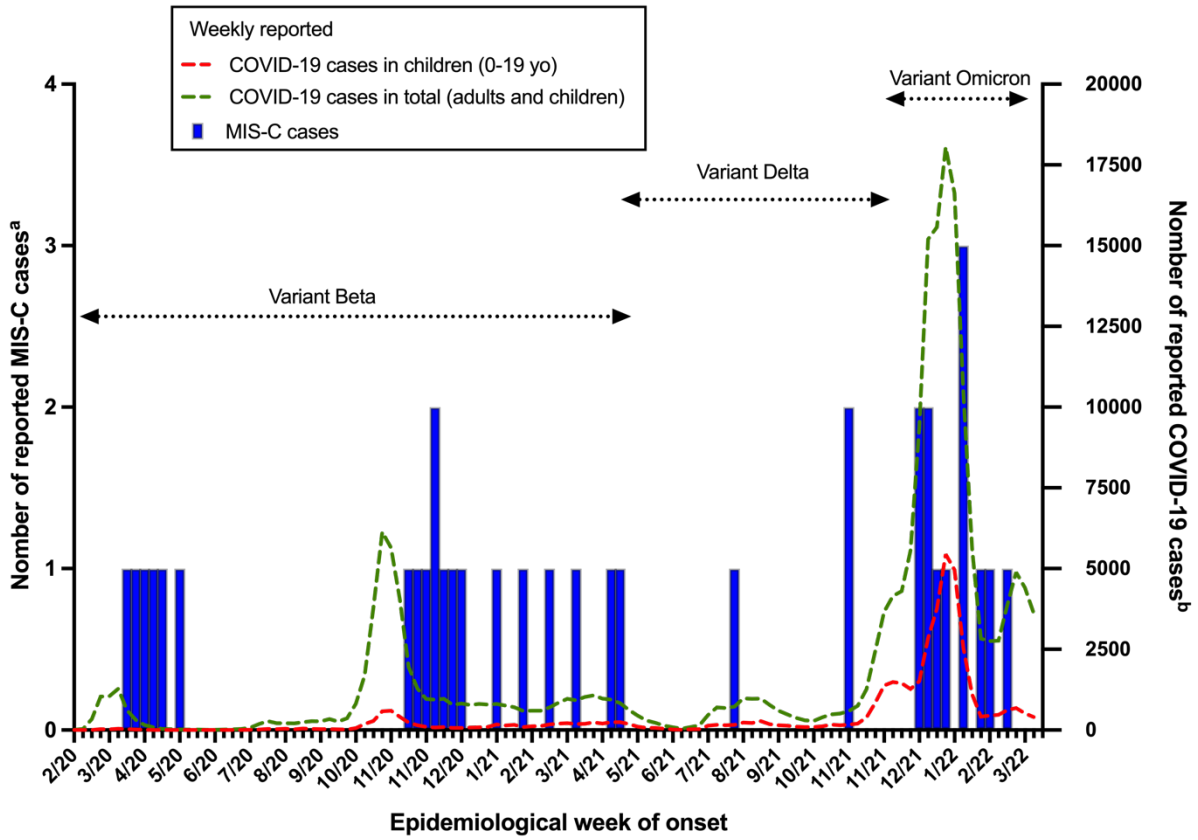

Figure 1. Reported cases of multisystem inflammatory syndrome in children by epidemiologic week of onset compared with cases of COVID-19 in children and youth aged 0-19 years and adults, Geneva between February 24, 2020 and March 28, 2022. Abbreviations: COVID-19 = coronavirus disease 2019; MIS-C = multisystem inflammatory syndrome in children. [a]Data source: MIS-C reporting Geneva University Hospitals. [b]Data source: Coronavirus disease 2019 surveillance system Geneva, Switzerland. SARS-CoV-2 variants: Variant Beta (B.1.351); variant Delta (B.1.617.2); variant Omicron (B1.1.1529)

2.0 MIS-C clinical presentation

MIS-C is a condition with clinical features affecting multiples organs, including the cardiac, gastrointestinal, mucocutaneous, respiratory, neurological, and hematological systems. Clinical signs and symptoms are summarized in Figure 2. Patients with MIS-C usually presented with persistent fever, very frequently associated with gastrointestinal symptoms such as abdominal pain, vomiting, and/or diarrhea. Children with MIS-C were also very often complaining of mucocutaneous symptoms reminiscent of KD, including conjunctivitis, skin rash, oral mucosal changes, or swollen extremities. The clinical presentation involves a continuum of severity ranging from mild symptoms to multi-organ failure with lethargy, hypotension and shock requiring admission to the pediatric intensive care unit (PICU)

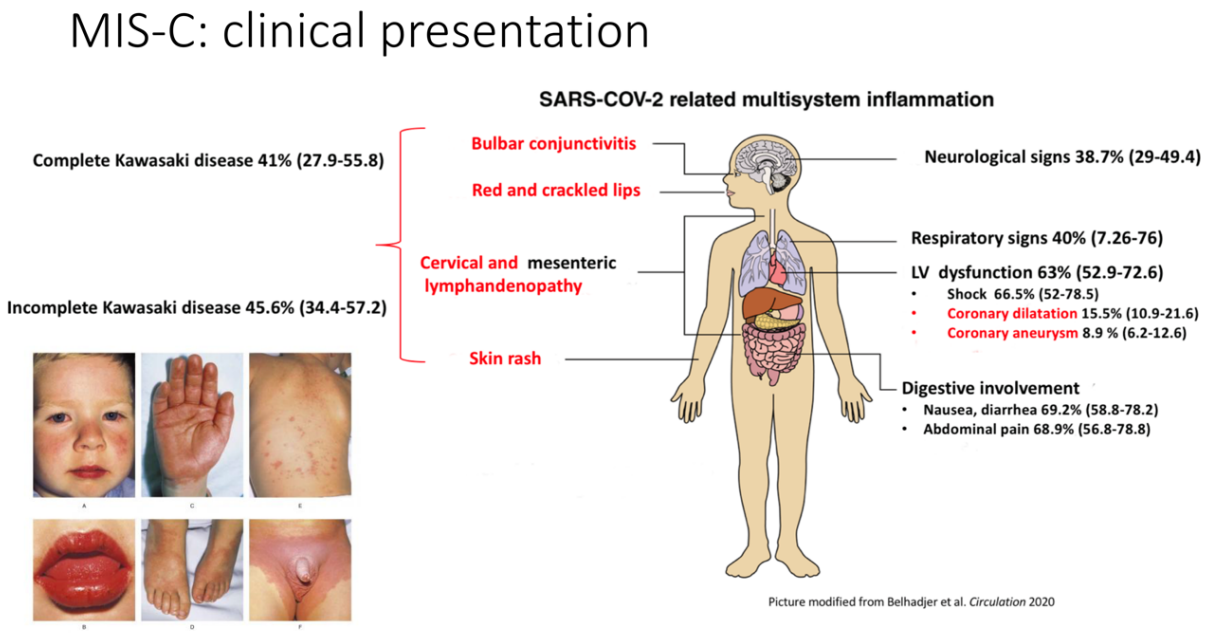

Figure 2. Schematic representation of the clinical signs of multisystem inflammatory syndrome in children. Red text indicates signs or symptoms consistent with Kawasaki disease; black text indicates signs that are rare in Kawasaki disease. Percentages and ranges in brackets are those from the current literature review.

2.1 MIS-C laboratory findings

As per definition, all MIS-C patients have elevated inflammatory markers including C-reactive protein (CRP), procalcitonin, erythrocyte sedimentation rate (ESR), and ferritin. They also present with hematological abnormalities including elevated white blood cell count with concomitant lymphopenia, low to normal platelet levels, elevated D-dimer, and elevated fibrinogen. Myocardial injury markers such as troponin and/or N-terminal-pro hormone B-type natriuretic peptide (NT-proBNP) are also frequently elevated in MIS-C patients (46, 51). Other common findings included hyponatremia, acute kidney injury, and hypoalbuminemia. It has been shown that some laboratory markers appear to correlate with severity of illness (46, 54). As demonstrated in Table 2, inflammatory and myocardial injury markers were significantly higher in MIS-C patients admitted at the University Hospital of Geneva with shock as compared to MIS-C patients admitted without shock.

Table 2. Laboratory data of patients reported with multisystem inflammatory syndrome in children (MIS-C)

Laboratory values	MIS-C (Total) (n = 17)	Non-Shock MIS-C (n = 7)	Shock MIS-C (n = 10)	P value[#]
WBC count (x10^9/L)	7.9 (6.5-10.35)	8.1 (7.2-8.7)	7.6 (5.6-13)	.845
Neutrophil count (x10^9/L)	6.9 (4.65-10.1)	6.9 (4.4-8)	6.8 (4.7-10.7)	.625
Lymphocyte count (x10^9/L)	0.6 (0.2-1.2)	0.74 (0.5-2.2)	0.52 (0.16-0.83)	.118
Platelets (x10^9/L)	145 (113.5-207.5)	199 (151-235)	127 (100-146.8)	**.019**
CRP (mg/mL)	214(181-286)	198 (136-265)	233 (190-329)	.143
PCT(μg/L)	8 (1.7-19.3)	1.8 (1.1-5.5)	13.9 (7.4-43.9)	**.006**
Il-6 (pg/mL)	93.5 (34.2-229.4)	74.6 (3.9-94.4)	129.5 (88.8-928)	**.03**
TNF-α (pg/mL)	19.8 (11.2-28.7)	11 (4.7-13.9)	27.7 (20.4-43.2)	**.001**
INR	1.08 (1-1.17)	1.06 (1-1.25)	1.09 (1.04-1.16)	.660
PT (%)	86 (72.5-97.5)	80 (62-100)	85 (74.3-92.5)	.590
PTT (sec)	36.9 (31.9-40)	35.3 (31-37)	38.3 (34.1-41)	.241
Fibrinogen (g/L)	5.3 (5-7.4)	5.6 (5.1-8.1)	5.1 (4.5-6.5)	.101
D-dimers (g/L)	3924 (2071-7539)	1969 (1527-2677)	6433 (3908-9178)	**.002**
Albumin (g/L)	30 (26-33)	32 (30-37)	27 (25-31)	**.028**
NT-proBNP (ng/mL)[a]	5422 (2591-21204)	2722 (1334-3978)	15724 (4217-32485)	**.02**
Troponin (ng/mL)	65 (31-214)	133 (35-199)	51 (27-246)	.696

Data are presented as median (interquartile range). CRP = c-reactive protein; PCT = procalcitonin; IL-6 = interleukin-6; TNF-α = tumor necrosis factor α; NT-proBNP = N-terminal probrain natriuretic peptide; PT = prothrombin time (percentage activity); PTT = partial thromboplastin time; INR = international normalized ratio. [#]Non-shock MIS-C vs shock MIS-C, Mann-Whitney U test. [a] Missing NT-proBNP data in 2 patients in the Non-shock MIS-C group and 1 patient in the Shock-MIS-C group.

2.2 MIS-C cardiovascular manifestations

Cardiac involvement occurs in up to 67-80% of children with MIS-C and represents a key feature of this new post infectious proinflammatory syndrome that helps to distinguish it from other proinflammatory syndrome in children, such as hemophagocytic lymphohistiocytosis, toxic shock syndrome or Kawasaki Disease (55). The most frequent cardiac manifestations reported included ventricular dysfunction, valvular regurgitation, coronary artery dilatation, arrhythmia, and conduction abnormalities.

2.2.1 Ventricular dysfunction

Several review of cardiac involvement in children with MIS-C reported ventricular dysfunction in 33 to 58% patients (55-58). Main echocardiography findings were evidence of both systolic and diastolic dysfunction with hypokinesia of the left ventricular wall and interventricular septum, left ventricular dilatation, atrioventricular valve regurgitation, and pericardial effusion. A reduction in left ventricular ejection fraction (< 55%) was reported in 34-50% of children with MIS-C (55-57, 59-62) and echocardiographic strains studies indicated abnormal ventricular strains in 11.4% of MIS-C patients (61). Abnormalities of diastolic function as evaluated by spectral and tissue doppler and strain were also described in MIS-C patients (61, 62). Interestingly, some MIS-C patients presented persistent abnormal ventricular strain associated with evidence of diastolic dysfunction after normalization of left ventricular ejection fraction (60, 61). Investigations of MIS-C patients during the acute phase by cardiac magnetic resonance (CMR) revealed signs of diffuse myocardial hyperemia and edema with hyperintensity in T2-short tau inversion recovery (STIR) sequences in 33-50% of scanned patients. Late gadolinium enhancement as evidence of myocardial fibrosis was uncommon during the acute phase of MIS-C and noted in 0-14% of scanned patients (56, 62, 63). Strain abnormalities were also observed on CMR in children presenting reduced left ventricular ejection on echocardiography (62). Cardiac biomarkers including troponin T and NT-proBNP were elevated in a large proportion of children with MIS-C and cardiac abnormalities on echocardiography and/or CMR. NT-proBNP levels were associated with the severity of myocardial injury in MIS-C patients (64, 65).

2.2.2 Coronary artery abnormalities

MIS-C, initially name Kawasaki-like disease because of the similar clinical picture with mucocutaneus involvement and conjunctivitis, shares another important clinical feature with KD that is the development of coronary dilatation, which has been reported in approximately 8-26% of MIS-C patients (55, 56, 66-68). The coronary artery dilatation is most often mild, but they are reports of children with MIS-C who developed severe coronary artery dilatation and, in rare cases, aneurysm (43, 45, 69). Although the presence of coronary artery dilatation has been described in MIS-C children with all type of clinical presentation/phenotype, it appears that the development of coronary artery abnormalities is more common in male children presenting with mucocutaneus and conjunctival involvement (54).

2.2.3 ECG abnormalities

ECG abnormalities were seen in 28-67% of MIS-C patients on admission (70) and the most common findings were low QRS amplitude and repolarization changes (abnormal ST- or T-wave segment) (51%) followed by first -degree block with prolonged PR interval (6.3-25%), second-or third-degree heart block (7%), QT prolongation (28%) or QRS prolongation (4%).

3.2.4 Pathogenesis of cardiovascular involvement in MIS-C

The pathogenesis of the cardiovascular manifestations in children with MIS-C remains not completely elucidated but appears to be likely multifactorial. The presence of elevated cardiac enzymes may indicate cardiomyocyte injury with a clinical picture of myocarditis caused by direct virus-mediated myocardial damage and/or host systemic immune response. Autopsy reports in MIS-C patients are limited but have demonstrated the presence of diffuse interstitial inflammation involving the endocardium, myocardium and pericardial with areas of myocardial necrosis. Interestingly SARS-CoV-2 could be detected in all of those patients in the lungs, heart and kidney as well as in endothelial cells from the heart and brain by one method (RT-PCR, immunohistochemistry or electronic microscopy) despite negative nasopharyngeal PCR testing for SARS-CoV-2 (71, 72).

However, some MIS-C patients may present with severe distributive shock and multiple organ failure requiring fluid resuscitation, inotropic support, and for the most severe cases

mechanical ventilation and extracorporeal membrane oxygenation (ECMO) but only mildly elevated troponin and normal or only slightly decreased ventricular function. This mode of presentation suggests that endothelial dysfunction may be involved in the cardiovascular manifestations of MIS-C. Indeed, the endothelium plays a major role in the regulation of vascular tone, vascular remodeling, immunity, inflammation, and platelet aggregation. It has been shown that the endothelium can be damaged with altered function in context of inflammation (73) and that endothelium dysfunction contributes to COVID-19 associated vascular inflammation and coagulopathy (74). The endothelial glycocalyx, which covers the luminal surface of endothelial cells, plays a key role in the regulation of inflammation and vascular permeability. Injury to the endothelial glycocalyx has been demonstrated in trauma and septic patients (75-77) and also recently in adult patients with severe COVID-19 (78, 79). In a recent study, we have demonstrated that children admitted with MIS-C presented signs of glycocalyx degradation with elevated levels of syndecan-1 in the blood and both heparan sulfate and chondroitin sulfate in the urine (Figure 3a) (65). The degree of glycocalyx shedding was more elevated in children admitted with MIS-C and shock than in children admitted with MIS-C without shock (Figure 3a). Glycocalyx degradation decreased overtime with normalization of circulating syndecan-1 levels at a median time of 100 days after symptoms onset (Figure 3b). Furthermore, the degree of glycocalyx shedding correlated with the level of circulating proinflammatory cytokine TNF-α (Figure 3c). In line with our findings, Ciftel et al. recently demonstrated the presence of endothelial dysfunction with lower flow-mediated dilatation in MIS-C patients that correlated with reduced aortic strain, aortic distensibility, and reduced ejection fraction (80).

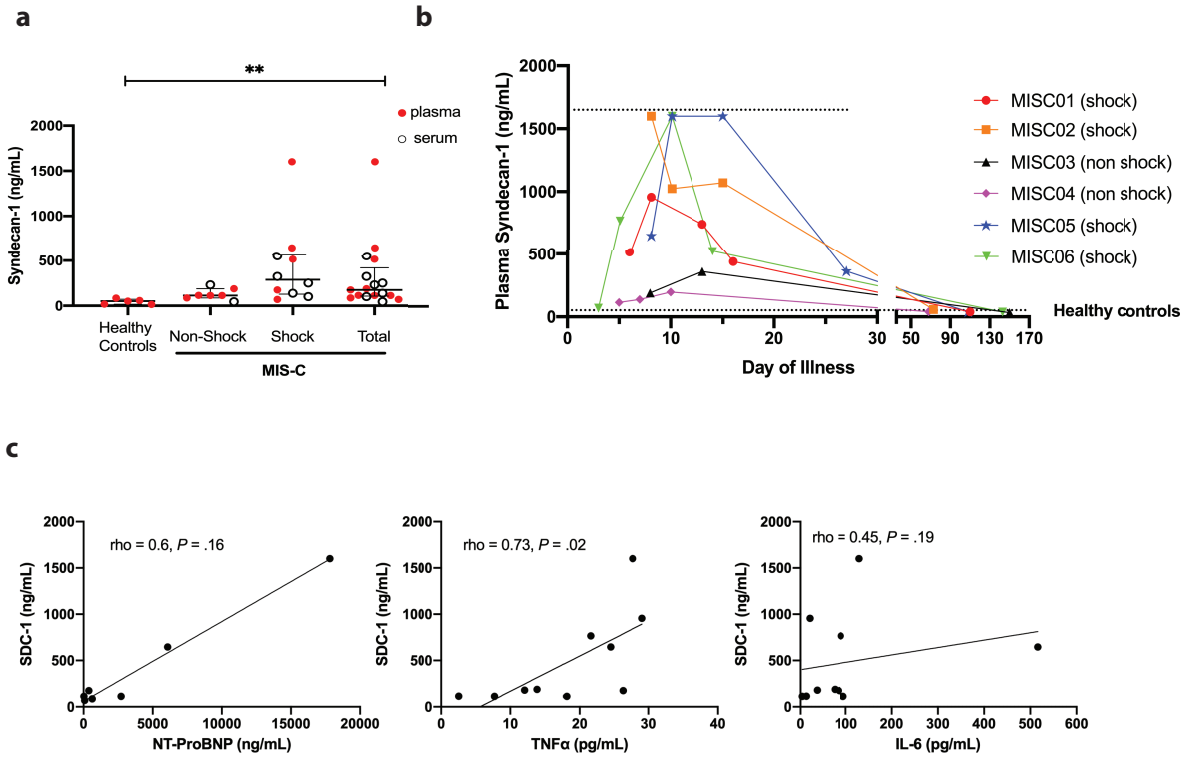

Figure 3: Syndecan-1 concentration increases in children with MIS-C**.** (**a**) Admission level of syndecan-1 (ng/ml) in healthy, MIS-C without shock and MIS-C with shock patients. Data represent median with interquartile range of plasma and serum samples. ***P* < 0.01, Mann-Whitney test (non-shock MIS-C vs shock-MIS-C) and (healthy controls vs total MIS-C patients). Black open circle = serum sample and red full circle = plasma sample (**b**) Time course of syndecan-1 plasma concentration in children admitted with MIS-C since hospital admission. The limit of detection of the ELISA is represented by a dotted line. Control data are the average of three control samples. (**c**) Correlation of plasma syndecan-1 with NT-proBNP, IL-6, and TNF-α. Spearman correlation test. IL-6 = interleukin-6; MIS-C = multisystem inflammatory syndrome in children; NT-proBNP = N-terminal probrain natriuretic peptide. TNF-α = tumor necrosis factor α.

Although the exact mechanism of the different cardiovascular manifestations of MIS-C remains to be determined, its transient nature, with rapid recovery of LV systolic function and resolution of shock within 1 to 2 weeks of diagnosis, suggests that cardiovascular dysfunction may be secondary to cytokine milieu, systemic inflammation, and acute stress cardiomyopathy rather than a direct virus cytotoxic effect.

2.3 MIS-C coagulopathy

Patients with MIS-C presented typically abnormalities in their coagulation cascade with elevated fibrinogen and D-dimers (43, 53) . Coagulation profiles performed in MIS-C patients using rotational thromboelastometry (ROTEM) revealed a prothrombotic state with

increased clot strength and slower fibrinolysis that correlated with D-dimer levels, peak platelet count, and erythrocyte sedimentation rate (81, 82). Derangement of hemostasis characterized by a prothrombotic state with increased thrombin generation and decreased fibrinolysis has been described in adults infected with SARS-CoV-2 (83) and was associated with an increased risk for thromboembolic while receiving therapeutic or prophylactic anticoagulation (84). Despite similar prothrombotic state, the risk of a thromboembolic events in children with MIS-C as compared to an adult with COVID-19 appear to be significantly lower with reported rate of thromboembolism of 3-6.5% in children with MIS-C (53, 85, 86) as compared to 20-40% in adults with severe COVID-19 (87). In absence of good data on the overall risk of thrombosis in children with MIS-C, guidelines for the management of thromboprophylaxis in patients with MIS-C were developed primarily based on evidence in analogous conditions such as KD and adult COVID-19. A phase 2 multi-center clinical trial is under way to determine the safety, dose requirements, and enoxaparin thromboprophylaxis in children hospitalized with SARS-CoV-2 related illness, including MIS-C (88). In the meantime, current guidelines suggest considering prophylactic anticoagulation based on the patient's risk for venous thromboembolism. Following factors were associated with an increased baseline risk for venous thromboembolism: patients > 12 years, altered mobility, obesity, known thrombophilia, history of thrombus, patients in critical condition, and presence of markedly elevated plasma D-dimer levels (eg, $\geq$ 5 times the upper limit of normal values)(89). Furthermore, considering the significant inflammatory vascular injury in MIS-C and as per analogy with KD, it is also recommended to start a treatment with low dose aspirin in all children with MIS-C that have no active bleeding or significant bleeding risk.

2.4 MIS-C gastrointestinal manifestations

Gastrointestinal (GI) signs and symptoms represent prominent features of MIS-C and are reported in 84-92% of MIS-C patients (53). Interestingly up to 29% of MIS-C patients had consulted within 7 days before hospitalization at an emergency room with fever and GI symptoms mimicking typical viral gastroenteritis with conservation of good general state and absence of other organ involvement (90). When abdominal imaging studies (abdominal sonography and CT) were performed, the most frequent findings were inflammatory bowel changes with diffuse bowel wall thickening, including marked terminal ileitis, mesenteric lymphadenitis, and ascites (90). Figure 4 illustrates inflammatory bowel changes and

mesenteric lymphadenitis found on abdominal CT in one MIS-C patient admitted at the University Hospital of Geneva.

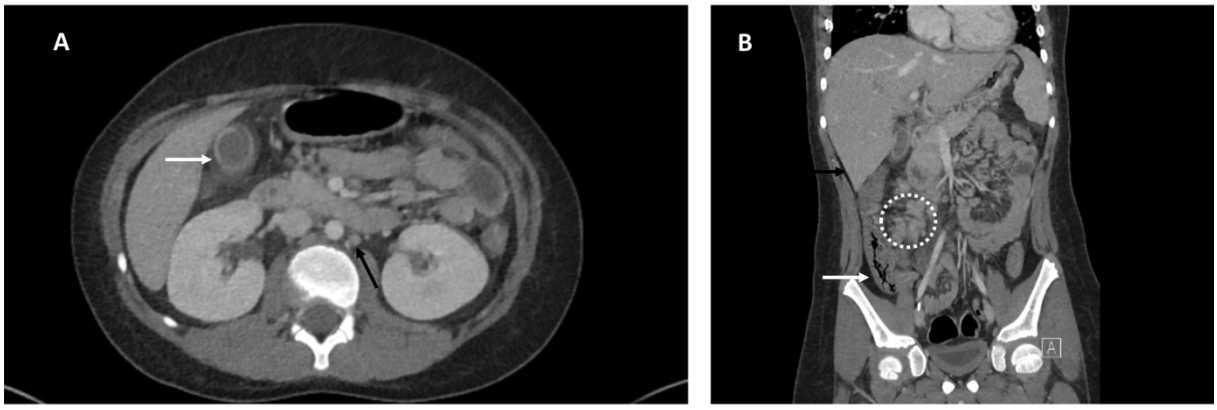

Figure 4. A: Axial CT with IV contrast in a MIS-C patient demonstrating right colon wall edema (white arrow). B: Coronal reconstruction from CT with IV contrast demonstrating mural thickening of the terminal ileum (white arrow) and mesenteric and retroperitoneal lymphadenopathy (white circle).

Differentiating acute appendicitis, viral gastroenteritis or inflammatory bowel disease from abdominal symptoms of MIS-C may be difficult both clinically and on abdominal imaging studies in particular abdominal CT given that frank appendiceal and right fossa inflammation or bowel wall thickening can be seen in respectively acute appendicitis and inflammatory bowel disease and MIS-C (91). There are reports indicating that up to 10% of children with final diagnosis of MIS-C were surgically explored for suspected appendicitis, highlighting the diagnostic challenge of this new pediatric syndrome mimicking numerous common pediatric diseases. The involvement of other organ system, such as the cardiovascular system or the presence of mucocutaneous symptoms with elevated inflammatory and myocardial injury biomarkers such as D-dimer, fibrinogen, and troponin may help to further distinguish MIS-C from surgical and inflammatory abdominal pathologies.

The mechanism for the GI manifestations in MIS-C remains unclear. It has been shown that a high density of ACE2 receptors is present on the surface of the absorptive enterocytes of the small intestine and the colon as well as on the surface of vascular epithelium, which may explain the GI symptoms in both adult and pediatric patients with acute SARS-CoV-2 infection. However, SARS-CoV-2 is rarely detected in both stool and ileocolic biopsy samples

from MIS-C patients by conventional methods. Interestingly, microscopic examination of ileocolic specimens from MIS-C patients found marked transmural lymphocytic inflammation and focal acute enteritis but also numerous venous microthrombi distributed in all layers of the ileum as well as signs of arteritis (92). Despite the absence of morphological evidence of acute bowel ischemia, it is possible that the vascular involvement in the gastrointestinal systems with presence of microthrombi and vasculitis in response to overwhelming inflammation may explain in part the gastrointestinal manifestations in MIS-C. In a recent study, Yonker et al suggested another potential mechanism that links the gastrointestinal symptoms and the severe inflammatory response in MIS-C (93). In their study, they were able to demonstrate by PCR in stool samples the presence of SARS-CoV-2 in the GI several weeks after initial SARS-CoV-2 infection or exposure. Dysbiosis caused by the presence of SARS-CoV-2 in the GI tract induced the release of zonulin, an important regulator of intestinal permeability, into the circulation resulting in the loss of GI mucosal barrier. As a result of the loss of gut integrity, circulating SARS-CoV-2 antigens, in particular SARS-CoV-2 spike, S1 and nucleocapsid antigens, could be detected in these MIS-C patients, which may be driven the hyperinflammatory responses of MIS-C. The administration of zonulin antagonist, larazotide to a patient with severe and refractory MIS-C induced rapid clinical improvement with defervescence and reduction of SARS-CoV-2 antigenemia and proinflammatory markers. These data support the role of intestinal leakage of SARS-CoV-2 antigens in the pathogenesis of MIS-C by triggering and maintaining a profound proinflammatory response.

2.5 MIS-C neurologic manifestations

Although gastrointestinal and cardiovascular symptoms were among the most frequently reported symptoms in MIS-C, 6-58% of patients presented also with neurological symptoms, potentially life-threatening in some cases, affecting both the central and peripheral nervous system (53, 94-96). The most frequent reported neurological signs in MIS-C patients were encephalopathy with behavior changes and altered level of consciousness (88%), hallucinations (36%), headaches or meningism (40%), seizures, peripheral nervous system involvement (40%), and focal central nervous system (CNS) involvement (ataxia, stroke) (97). Cerebrospinal fluid (CSF) analysis in those patients were frequently normal with absence of SARS-CoV-2 detected by PCR. The electroencephalogram (EEG) showed nonspecific focal or

general background slowing consistent with encephalopathy or presence of epileptic charges in some patients (94). Up to 76 % of MIS-C patients presented abnormal findings on cerebral magnetic resonance imaging (MRI) or CT. Although some children presented signs of stroke or diffuse cerebral oedema, a typical finding on cerebral imaging in MIS-C patients was changes involving the splenium of the corpus callosum consistent with mild encephalopathy with reversible splenial lesions syndrome (RESLES) (95-100). Interestingly RESLES had also been observed in children with KD (101, 102) as well as in other viral (103, 104) and inflammatory encephalopathies (105, 106). RESLES represents intramyelinic edema in the corpus callosum as a result of cytokine mediated glutamate release. Although most of the neurological symptoms were self-limiting and improved over time, some children presented persistent neurological symptoms, with reports of children developing devastating neurological sequelae or dying as the result of their neurological complication (96). Underlying pathogenesis of neurological manifestations and complications in MIS-C remained unclear. The current proposed mechanism, extrapolated from animal and basic science studies, include direct viral infection of the nervous system and its vasculature, or a cytokine driven neuroinflammation either secondary to local infection and/or secondary to systemic infection. Although animal models have demonstrated the expression of ACE2 and transmembrane serine protease 2 (TMPRSS2) on neurons and glial cells (107), data supporting the hypothesis of a direct viral effect upon the central nervous system is poor with the identification of SARS-CoV-2 in the brain made technically difficult and limited to the detection of the virus by PCR in the CSF (96, 97). In a recent study, Sa et al. have shown that children admitted with MIS-C with neurological symptoms had significantly higher systemic inflammatory markers than children with MIS-C without neurological features (94). Furthermore, they demonstrated that among the children with neurological features, peak inflammatory markers and D-dimer levels measured during the acute phase were higher in children with persistent neurological sequalae at 3-month follow-up as compared to children with complete neurological recovery. These findings support the hypothesis of a neuroinflammation driven by a systemic para or postinfectious immune-mediated phenomenon.

3.0 MIS-C treatment

In MIS-C patients, goals of treatment are to restore immune homeostasis in patients with life-threatening manifestations and to prevent long-term sequelae, that may include coronary artery abnormalities, myocardial fibrosis or central or peripheral nervous systems dysfunction. Both intravenous immunoglobulin (IVIG) and glucocorticoids represent the most used immunomodulatory medications reported in MIS-C patients (43, 45-47, 51, 53, 108-113). In the absence of randomized controlled clinical trials, the paucity of high-quality evidence for management results in significant variability across clinical practice worldwide. To provide guidance to healthcare providers taking care of MIS-C patients numerous expert-based guidelines extrapolated from other inflammatory or rheumatic conditions with similar clinical presentations (eg, KD, cytokine release syndrome, and macrophage activating syndrome) started to be published (114). In December 2020, a Swiss experts consensus guideline was developed as "Best Practice Recommendations for the Diagnosis and Management of Children with Pediatric Inflammatory Multisystem Syndrome Temporally Associated with SARS-CoV-2 (PIMS-TS; Multisystem Inflammatory Syndrome in Children, MIS-C) in Switzerland" to standardize management in Switzerland (115). The Swiss Best Practice guidelines was applied in Geneva in December 2020 and Table 3 describes clinical characteristics, biologic data, treatments, and outcome in MIS-C patients admitted before (pre-protocol group) and after the implementation of the guidelines (post-protocol group). Overall, there were no significant differences in clinical characteristics and laboratory values (including proinflammatory and myocardial injury markers) at admission between the two groups.

Table 3 Comparison between patients admitted with multisystem inflammatory syndrome in children (MIS-C) before and after the introduction of MIS-C best practice guidelines

	Overall (n = 20)	Pre-protocol group (pre-MIS-C guidelines) (n = 6)	Post-protocol group (post-MIS-C guidelines) (n =14)	P value*
Patient's characteristics				
Age	10 (8-13)	10.5 (10-11.5)	9 (6.5-13)	.587
Male	16 (80)	5 (83.3)	11 (78.5)	1.0
BMI	17.3 (14.8-23.8)	18 (15.8-26.7)	16.6 (14.2-22.1)	.334
Comorbidities	0	0	0	1.0
Day of illness at admission	6 (5-6.8)	7 (4.8-8)	5.5 (4.8-6)	.190
Laboratory tests				
CRP (mg/mL)	239.4 (189.3-290.5)	233 (172.5-280.8)	222.4 (184.8-304.6)	.741
PCT (μg/L)	5.9 (1.7-14.8)	12.1 (2.3-61.7)	5.53 (1.64-11.4)	.219
Il-6 (pg/mL)	92.6 (20.8-146.1)	85.5 (61.1-226.1)	94.4 (9.9-201.6)	1.0
TNF-α (pg/mL)	19.7 (9.9-27.7)	28.4 (11.3-54)	18.1 (8.8-22)	.136
D-dimers (g/L)	4093 (1989-7071)	4247 (3924-7478)	2893 (1908-7227)	.355
Albumin (g/L)	26 (24.3-28)	24 (22.5-29)	26.5(25-30)	.229
Myocardial injury markers				
NT-proBNP (ng/mL)	5625 (2460.5-24594)	17814#	5422 (2025-19637)	.231
Troponin (ng/mL)	88 (30.5-192.3)	182.5 (43.75-251.5)	61 (27.3-168.3)	.322
Cardiac involvement,				
LVEF < 55%	8 (40)	2 (33)	6 (43)	1.0
Coronary abnormalities	4 (20)	2 (33)	2 (14)	.549
Treatment				

Admission to ICU	18 (90)	4 (66.6)	14 (100)	.079
Invasive mechanical ventilatory support	2 (10)	2 (33.3)	0 (0)	.079
Dialysis	1 (5)	1 (16)	0 (0)	.3
Vasoactive medications	12 (60)	4 (66.6)	8 (57)	1.0
IVIG	17 (85)	3 (50)	14(100)	**.018**
Corticosteroids	17 (85)	3 (50)	14 (100)	**.018**
Biologics	6 (30)	4 (66.6)	2 (14)	**.037**
Outcome				
ICU length of stay (days)	4 (1.8-7)	10 (7.5-13.3)	2 (1-5)	**.003**
Hospital length of stay (days)	7 (6.3-9.8)	9.5 (8.5-23.5)	7 (5.8-8.3)	**.015**
Survival to discharge	20 (100)	6 (100 %)	14 (100 %)	1.0

Data are presented as median (interquartile range) for continuous variables or No (%) for binary variables. CRP = c-reactive protein; PCT = procalcitonin; IL-6 = interleukin-6; TNF-α = tumor necrosis factor α; NT-proBNP = N-terminal probrain natriuretic peptide; LVEF = left ventricular ejection fraction; IVIG = intravenous immunoglobulin NA = non-applicable. [#] Data available only in 3/6 patients. [*]pre-protocol group vs post-protocol, Mann-Whitney U test or Fisher exact test

However, the application of the best practice recommendation for the management of MIS-C had a significant impact on the treatment regimens among the two groups. All the patients in the post-protocol group received a single dose of IVIG and systemic corticosteroids as compared to only 3 patients from the pre-protocol group (100% vs 50%; *P* = .018). In addition, biologics (Anakinra, a recombinant human IL-1 receptor antagonist or Tocilizumab, an IL-6 receptor antagonist) were used only in two patients from the post-protocol group as compared to 4 patients from the pre-protocol group (14% vs 67%; *P* = .037).

Both ICU and total hospital LOS were significantly shorter for patients from the post-protocol group as compared to patients from the pre-protocol group (2 days [IQR 1-5 days] vs 10 days [IQR 7.5-13.3 days]; *P* = .003) and 7 days [IQR 5.8-8.3 days] vs 9.5 days [IQR 8.5-23.5 days]; *P* = .015). There was also a lower proportion of patients from the post-protocol group that required invasive mechanical ventilatory support or dialysis as compared to patients from the pre-protocol group (0% vs 33%; *P* = .079 and 16% vs 0%; *P* = .3). Figure 5 illustrates the effect of IVIG, systemic corticosteroids and biologics on the fever and requirement for vasoactive support among the 8 patients from the post-protocol group presenting with the most severe form of MIS-C. We observed that in four patients (MISC10, MISC11, MISC12, and MISC13) vasoactive support could be stopped within 72 hours after IVIG injection and corticosteroids therapy without recurrence of fever. In patient MISC18, treatment was started with a delay because of initial suspicion of toxic shock syndrome. Once the MIS-C diagnosis was made and MISC18 patient received IVIG and corticosteroids, the vasoactive support could be weaned within 24 hours without fever recurrence. Three patients (MISC07, MISC08 and MISC16) presented persisting fever and hemodynamic instability that failed to respond to first line therapy with IVIG and corticosteroids. They all required, as per protocol, a second line treatment with Anakinra (MISC7 and MISC08) or a second dose of IVIG (MISC16) to which they all responded well.

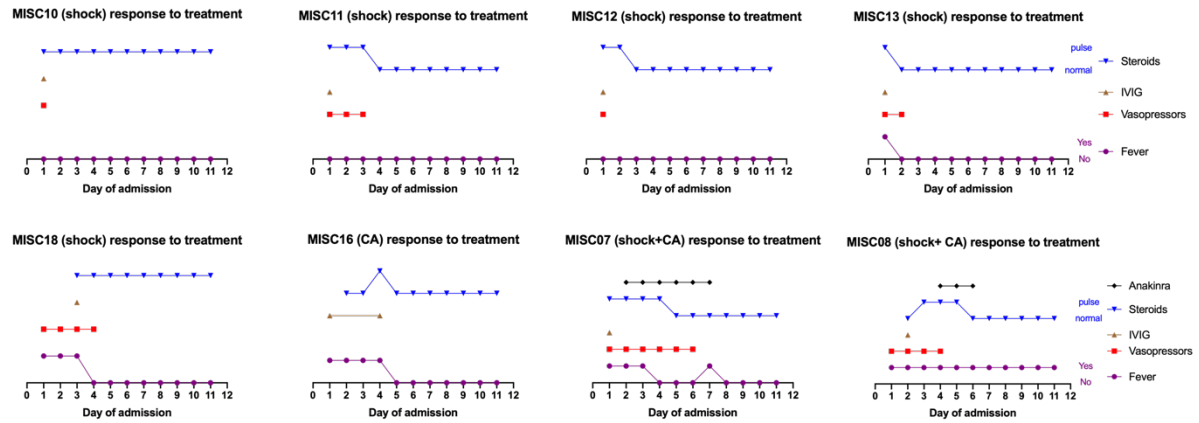

Figure 5: Timeline of response to immunomodulatory treatments on fever and requirement for vasopressors in 8 MIS-C patients from the post-protocol group. Time in days since admission is shown on the horizontal axis. The vertical axis shows the response to different immunomodulatory treatment, including IVIG (brown triangle), steroids (inverted blue triangle), biologics (rotated black square) on fever (purple circle) and requirement for vasopressors (red square). CA = coronary artery abnormalities; IVIG = intravenous immunoglobulin; MIS-C: multisystem inflammatory syndrome in children.

Current data from the literature on the efficacy of the different immunomodulatory treatments (IVIG, systemic corticosteroids, and biologics) are limited to three recent large retrospective cohort studies (59, 116, 117). McArdle *et al.* compared IVIG versus IVIG plus glucocorticoids and IVIG versus glucocorticoids alone in a large international cohort study of MIS-C patients. They found no differences on the composite primary outcome of inotropic support or mechanical ventilation by day 2 or later or death among the different treatment regimens (117). These results are different from the findings of two other cohort studies, which found a beneficial effect of IVIG plus glucocorticoids as compared to IVIG alone, with a lower rate of treatment failure (defined by the persistence of fever at day 2) (116), and requirement for second line treatment and a lower rate of cardiovascular dysfunction on day 2 (59). Many factors may explain the divergent findings among these three studies: differences in the study populations with various degree of MIS-C severity, time-period of the study with variations in circulating viral variants, and differences in the criteria used for the initiation and escalation of the different immunomodulatory treatments. In response to the urgent need of data on the effectiveness of the different immunomodulatory drugs and treatment regimens for MIS-C patients, two pediatric randomized multicenter clinical trial, the Swissped RECOVERY (NCT04826588; Swissped RECOVERY) and the United Kingdom RECOVERY

(NCT04381936;UK RECOVERY) were recently launched in 2021 to assess the effectiveness of IV methylprednisolone and IVIG in hospitalized MIS-C patients. Swissped RECOVERY was inspired by RECOVERY trial running in the UK. The protocol (including inclusion/exclusion criteria and primary outcome of hospital length of stay) was similar and the main difference between the two studies lies in the different randomized treatment arms with 3 treatment arms in the UK RECOVERY (standard of care vs IVIG vs methylprednisolone) and 2 treatments arms in the Swissped RECOVERY (IVIG vs methylprednisolone, but no standard of care arm). While waiting the results of those two clinical trials, the current available evidence on the best management of children admitted with MIS-C consists of a stepwise approach with the use of a dual therapy with both intravenous immunoglobulin and glucocorticosteroids considered as a first-line treatment for most hospitalized patients, in particular the ones with the most severe presentation who present signs of shock and/or severe encephalopathy (114). This is based on the recent evidence from cohort studies that suggest a potential benefit of the combined therapy with IVIG plus glucocorticosteroids to lower the risk of treatment failure, improve left ventricular function and the need for adjunctive immunomodulatory therapies (59, 116-118). For MIS-C patients who are considered refractory to this first line of immunomodulatory treatment, with persistent fevers and/or significant end-organ dysfunction within the first 24 hours of treatment, intensification therapy is recommended with either high-dose (10-30mg/kg/day) glucocorticoids or biologics such as anakinra or infliximab (114). Anakinra and Infliximab (TNF-α inhibitor) represent two proinflammatory cytokine inhibitors that are frequently used as adjunct therapies or steroid-sparing agents for MIS-C (46, 53, 113, 119-124).

Vaccination against SARS-CoV-2 started worldwide in December 2020 (125) and in Switzerland the vaccination campaign was organized in 3 phases: initially only for adults (as of December 2020), then open to teenagers > 12 years old (as of June 2021), and finally open to children 5-11 years old (as of December 2021). Since the beginning of the vaccination campaign among adolescents, two recent publications have shown that COVID-19 mRNA vaccination was highly protected against MIS-C among fully vaccinated adolescents aged 12-18 yo who received 2 doses (126, 127). In our center in Geneva, 100% of all patients admitted to the hospital with a diagnosis of MIS-C were unvaccinated, which is consistent with the results of those two recent studies who reported that 95-100% of hospitalized MIS-C patients were either unvaccinated

or partially vaccinated. It is important to mention that cases of children who developed MIS-C after SARS-CoV-2 vaccination without evidence of SARS-Co-2 infection have been recently reported (128, 129). Although this possible complication of the COVID-19 vaccination needs to be closely monitored, it remained however a very rare complication with a reporting rate lower than 1 per million vaccinated individuals aged 12-20 years and a reassuring clinical course with all patients being able to be discharged home.

4.0 MIS-C immunopathogenesis

MIS-C is characterized by a severe and uncontrolled hyperinflammatory syndrome with multiorgan involvement appearing weeks after SARS-CoV-2 infection and sharing clinical features with TSS, KD, macrophage activating syndrome (MAS), and cytokine release syndrome. Although its pathogenesis remained unclear, recent studies were able to bring some insight into its immunopathogenesis by performing a throughout immune profiling and immunophenotyping in MIS-C patients and in adults and children with acute SARS-CoV-2 infection or children with KD using various techniques including proteomics, RNA sequencing, autoantibody arrays, and B cell receptor repertoire analysis

4.1 Biomarkers profile of MIS-C patients

Systemic inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-10, IL-17, IL-18, IFN-γ, monocyte chemoattractant protein 1 (MCP-1/CCL2), and interferon gamma-induced protein 10 (IP-10/CXCL10) were all significantly elevated in MIS-C patients as compared to healthy controls (130-132). When organizing these different biomarkers according to pathways we can see that the following pathways were highly activated in MIS-C patients : type II IFN signaling (IFN-γ, C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10), macrophage activation (IL-6, soluble tumor necrosis factor receptor I (sTNFRI), IL-10, soluble form of the IL-2 receptor (sCD25), IL-17, TNF-α, chemokine ligand 2 (CCL2), CCL3, CCL4, ferritin and IL-15), neutrophil activation (myeloperoxidase (MPO) and lactoferrin), and matrisome-related inflammation (matrix metalloproteinase-9, (MMP-9), soluble isoform of ST2 (sST2)/sIL-33R and chemokine fractalkine (CX3CL1) (133, 134). Proteomic characterization revealed different profiles allowing the differentiation of severe MIS-C from mild disease and from KD. Proteins involved in proteolysis, classical complement cascade,

coagulation, acute phase response, and inflammation (RP, S100A9, SAA1, SAA2, STAT3, FCGR3A, LBP, CD163, ORM1, SERPINA1, SERPINA3, TIMP1, TLN-1, VWF, various Igs, and components of the C1 complex of the complement system) were significantly more elevated in severe MIS-C patients as compared to mild MIS-C (135). Interestingly, MIS-C patients presented low levels of C-C motif chemokine 22 (CCL22), a homeostatic chemokine promoting regulatory T cell migration and function that is negatively regulated by IFN-γ (136, 137) as compared to healthy controls. The reduced regulatory T cell responses secondary to low CCL22 levels might promote uncontrolled inflammation in MIS-C. When reassessing these biomarkers overtime, it has been shown that most of them decreased during hospitalization in parallel with clinical improvement and after treatment with IVIG and/or glucocorticoids (133). Multiple components of the complement pathway, including complement proteins C9 and C5b-9, were also elevated in MIS-C patients with notably higher levels in patients with the most severe form of MIS-C (130).

When comparing MIS-C and KD biomarker profiles, both shared some similar protein profiles indicating similar pathogenic pathways; however MIS-C patients were distinguished by pathways involved in immune complexes and heart muscle, and significantly higher IFN-γ induced CXCL9 levels (138).

4.2 Cellular responses in MIS-C patients

Analysis of peripheral blood mononuclear cells (PBMCs) from MIS-C patients revealed neutrophilia and lymphocytopenia with reductions of $CD4^+$, $CD8^+$, γδT cell, and natural killer (NK) cells (132, 134, 139). Naive B cells and plasmablasts were increased and conventional dendritic cells (cDC), and plasmacytoid dendritic cells (pCD) were decreased in MIS-C patients as compared to healthy controls. A possible explanation for the characteristic lymphocytopenia with reduced number of lymphocytes subsets observed in MIS-C patients may be the result of lymphocytes extravasation and chemotaxis to affected tissues in response to cytokines and chemokines including CCL19, CXCL10 and CUB Domain Containing Protein 1 (CDCP1).

Immunophenotyping of T and B cells in MIS-C patients demonstrated increased activated neutrophils and monocytes with high CD64 expression and decreased antigen-presenting

ability among monocyte and dendritic cell population with reduced expression of HLA class II antigen presenting molecules and CD86, an important molecule for providing costimulatory signals to induce T cell activation. Although decreased in absolute number in MIS-C patients, NK cells however exhibited increased expression of cytotoxic genes with elevated levels of Granzyme A proteins (139). Monocytes and neutrophils were found to share upregulation of the alarmin-related S100A genes (*S100A8*, *S100A9* and *S100A12*) (139). A subset of proliferating $CD4^+$ T cells ($Ki67^+$) were identified in MIS-C patients by flow cytometry with downregulation of *CXCR5* gene but upregulation of *ICOS*, *PDCD1, MAF*, and *IL21* genes as well as chemokine receptor genes such as *CCR2*, *CXCR1*, and *CCR5.* Interestingly, there was a correlation between proliferating $CD4^+$ T cells ($Ki67^+$) and proliferating plasmablasts ($Ki67^+$). Furthermore, Vella et al, described the presence of highly activated vascular patrolling CX3CR1+CD8 T cells in MIS-C patients that were positively correlated with D-dimer levels and the need for vasoactive support suggesting a possible mechanism for the cardiovascular complications in MIS-C linking CX3CR1+CD8 T cells activation and vascular disease (140).

4.3 Autoreactivity and superantigen hypothesis in MIS-C patients

T-cell receptor (TCR) repertoire analysis of $CD4^+$ and $CD8^+$ memory T cells from children with MIS-C revealed richer and more diverse TCR repertoire in mild MIS-C as compared to severe MIS-C patients associated with significant skewing of the V-beta repertoire with increased expression of *TRBV11-2, TRBV24-1*, and *TRBV11-3* genes in severe MIS-C patients (139, 141). Interestingly, *TLRBV11-2* gene overexpression, which was HLA-type I associated, correlated with TNF-α, IFN-γ, IL-6 and IL-10 levels suggesting a possible link between TRBV11-2 expansion and the cytokine storm seen in severe MIS-C patients (141). Furthermore, a superantigen-like motif had been identified near the S1/S2 cleavage site on the SARS-CoV-2 spike protein (142). These recent findings support the superantigen hypothesis for the pathogenesis of MIS-C by which a superantigen-like motif within the SARS-CoV-2 spike glycoprotein is driving the activation and proliferation of *TRBV11-2* positive polyclonal T cells (141, 142).

4.4 Autoimmunity and tissue injury in MIS-C patients

All MIS-C patients had detectable anti-spike (S) IgG and IgA antibodies. But, unlike patients with acute SARS-CoV-2 infection, MIS-C patients lacked IgM antibodies consistent with the

hypothesis of a post infectious inflammatory syndrome that develops weeks after initial infection (130). When comparing the SARS-CoV-2 specific antibodies among MIS-C patients and convalescent adults after SARS-CoV-2 infection, severe MIS-C patients exhibited higher anti-spike IgG levels with distinct features. Indeed, SARS-CoV-2 specific antibodies measured in severe MIS-C patients exhibited increased phagocytic and function activity with enhanced monocyte-activating capacity suggesting a potential immune complex (IC)-based activity in MIS-C pathogenesis (143-145). In addition to the proinflammatory antibody profile, MIS-C patients presented also with an expansion of highly functional antibodies against multiple pathogens, some of them possibly related to KD such as Epstein-Barr virus (EBV) but also against measles, endogenous retrovirus, *Bordetella pertussis*, and *Staphylococcus aureus* (145). These findings point to a dysregulated humoral immune response driving a broad inflammation in severe MIS-C through non-specific amplification of functional pathogen-specific IgG driven immunity.

A striking finding in MIS-C patients in addition of the severe proinflammatory response is the multisystem involvement. Therefore, recent studies investigated the presence of autoantibodies in MIS-C patients (134, 135, 146). They discovered the presence of autoantibodies in MIS-C patients against several tissue-specific antigens from organs typically affected in MIS-C, such as the GI tract, cardiovascular system, skeletal muscle, and brain tissues. A list of some of the organ-specific auto-antigens reported in the recent literature are listed in Table 4.

Table 4. List of organ-specific auto-antigens measured in MIS-C patients

Organ	Name of the protein	Reference
Gastrointestinal tract	ATP4A	(135)
	SOX6	(135)
	FAM84A	(135)
	RAB-11FIP1	(135)
	MUC15	(134)
	TSPAN13	(134)
	SH3BP1	(134)
Cardiovascular system	PDLIM5	(135)
	EIF1AY	(135)
	Endoglin	(134)
	P2RX4	(134)
	ECE1	(134)
	MMP14	(134)
Skeletal muscle	RBM38	(135)
	TNNC2	(135)
Brain tissue	MAP9	(135)
	NAPB	(135)

In addition of tissue specific-autoantibodies, presence of antibodies typically found in autoimmune disease were also identified in MIS-C patients such as anti-La and anti-Jo1 antibodies, which are characteristic of respectively systemic lupus erythematosus, and Sjögren's syndrome and idiopathic inflammatory myopathies, respectively (134).

As already mentioned , MIS-C patients, and in particular patients with severe MIS-C, are characterized by clinical and biological signs of endothelial dysfunction with shock, severe capillary leak and elevated endothelial injury markers including syndecan-1, vascular endothelial growth factor (VEGF), soluble vascular cell adhesion molecule-1 (sVCAM-1/sCD106), and soluble E-Selectin (134, 139). Recent studies demonstrated not only the presence of autoantibodies targeting the endothelial cells but also the ability of antibodies collected from serum of MIS-C patients to activate *ex vivo* human cardiac microvascular endothelial cells in culture (139).

This recent evidence points to an autoimmune phenotype in MIS-C patients characterized by a dysregulated B cells response with autoantibody production, complement activation and myeloid-mediated inflammation. Immune complex-mediated tissue damage and cell death may then contribute to excessive antigenic drive and autoantibody production in a vicious circle. Furthermore, it has been shown that autoantibodies can promote a proinflammatory response through FcγR-mediated neutrophils stimulation and complement activation, which may represent a possible mechanism of the effect of IVIG described in MIS-C by neutralizing FcγR proteins on the surface of immune cells and inhibition of complement pathway (147). Cross talk between immune cells, in particular highly activated neutrophils, autoreactive B and T cells, and activated plasmablasts with increased survival ability, appear to play a significant role in the development and persistence of this vicious inflammatory circle identified in MIS-C patients.

4.5 Possible role of gut dysfunction as driving mechanism for autoreactivity and autoimmunity MIS-C patients

With the absence of SARS-CoV-2 detectable by reverse transcription PCR of the nasopharyngeal swab in most patients with MIS-C, the source and location of the superantigen driving the hyperinflammatory response in MIS-C remains unclear.

GI symptoms are very common in MIS-C patients and recent evidence suggest a possible role of the GI tract in the pathogenesis of MIS-C (148). Yonker et al. discovered the presence of SARS-CoV-2 RNA in the stool of MIS-C patients that persisted weeks after initial infection with evidence of gut microbial translocation with increased serum levels of lipopolysaccharide-binding protein (LBP) and CD14, and SARS-CoV-2 antigenemia(93). Interestingly, they also demonstrated that zonulin-dependent loss of gut integrity with intestinal leakage of SARS-CoV-2 antigens, in particular S1 known to have super-antigen-like properties, correlated with elevated antibody titers against S1 and TRBV11-2 skewing (148). These data suggest that gut dysfunction with increased permeability either as a direct result of SARS-CoV-2 viral direct toxicity or secondary to autoantibodies mediated-tissue injury may represent potential mechanisms for SARS-CoV-2 antigenemia that is fueling the hyperinflammatory response of MIS-C. The gut appears therefore as an interesting therapeutic target in MIS-C in addition of the systemic immunomodulatory treatments used so far.

5.0 MIS-C outcome

Most of MIS-C patients exhibit rapid clinical improvement within 48 hours after receiving one or several immunomodulatory therapies (e.g. IVIG, corticosteroids, and biologics) and can be discharged after a median hospital length of stay of 5-10 days(55, 149). Despite this rapid initial favorable evolution with a low mortality rate, recent reports start to emerge describing the short- and long-term impact of this severe hyperinflammatory syndrome on the recovery of the different affected organs and the quality of life for those children (58, 121, 150-152).

From the cardiovascular point of view, most MIS-C patients with LV systolic dysfunction at admission have a normalization of the LV systolic function by discharge (69%), with a normal function in all the patients at an eight-week follow-up consultation (150, 151, 153). Of the patients who presented with coronary abnormalities during hospital stay, a large proportion had resolution or improvement; only a small proportion (9%) were reported to have persistent coronary aneurysm at a 6-month echocardiographic follow-up (58, 151). Despite normalization of the LV systolic function during the follow-up echocardiography in almost all the patients, diastolic function remained abnormal in a small proportion of them (9%) at the 6-month follow-up (153). In line with the normalization of the cardiac function and coronary artery abnormalities, myocardial biomarkers (troponin and NT-ProBNP) as well as biomarkers of endothelial glycocalyx dysfunction (syndecan-1) were all normal by 3-6 months (58, 65, 151, 153).This rapid resolution of both cardiac function and coronary artery abnormalities in MIS-C patients represent another important difference with the clinical course of KD patients who often have persistent coronary artery aneurysm. This support the current hypothesis that cardiac manifestations in MIS-C are the result of the systemic inflammation with cardiac stunning rather than a direct immune mediated damage of the myocardium with progressive endovascular changes as observed in KD (61).

Persistent neurological symptoms were reported in up to 39% of MIS-C patients at 6 months with most common sequelae included muscular fatigue, proximal myopathy, dysmetria, abnormal saccades, and attention deficit. MIS-C patients presented also signs of anxiety

(31%), emotional lability (26%), emotional distress (7%), and sleep symptoms (42%) at the 6-month follow up (58, 152).

A small proportion of MIS-C patients (13%) are also reporting persistent abdominal symptoms at 6 months, most abdominal pain and diarrhea (58).

Although most of the MIS-C patients have recovered from initial severe illness, with normalized biochemical and functional outcomes, some patients have persistent organ-specific sequelae that may impact their functional abilities to return to school or resume a physical activity. In line with the current evidence supporting a multidisciplinary follow-up after PICU discharge for children admitted with a critical illness (154), these results highlight the importance of physical rehabilitation and mental health support for MIS-C patients. Indeed, the long-term functional and neuropsychological impact of MIS-C remain unknown and warrant further investigation.

Conclusion

MIS-C represents a perfect illustration of the critical role played by the different national and international surveillance systems. The case definition of this new hyperinflammatory syndrome affecting children were published by the RCPCH (50), CDC (49), and the WHO (48) only a few days after the publication of the first report of a cluster of patients admitted in shock with elevated proinflammatory markers and clinical symptoms similar to Kawasaki disease mid-Avril 2020 (43). The rapid creation of a case definition for this new pediatric disease associated with both national and international report systems for MIS-C allowed the creation of large international patient registries (Figure 6).

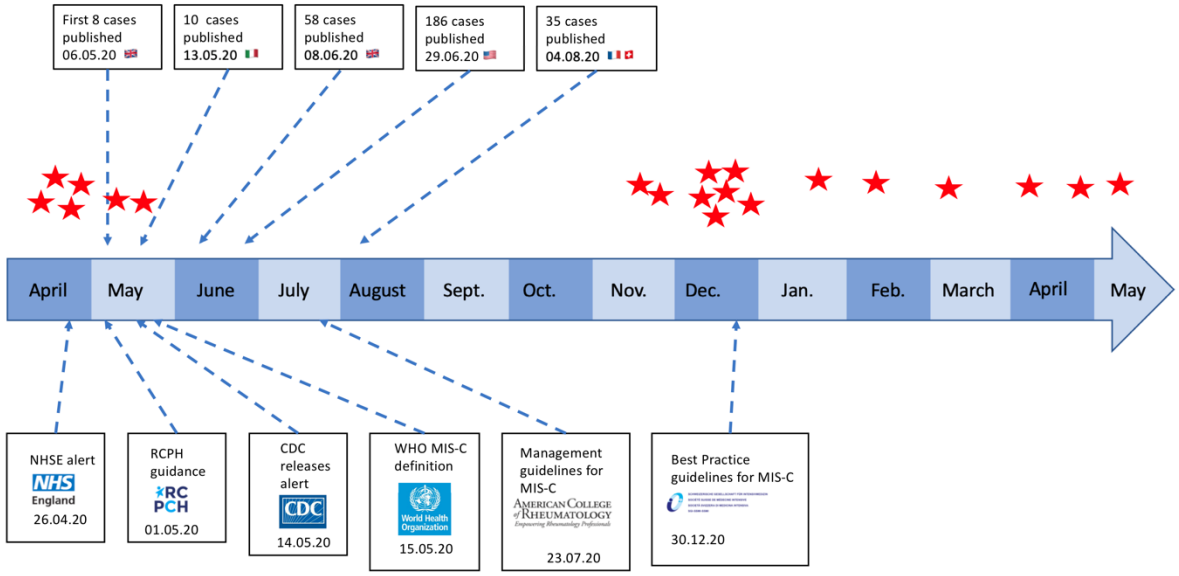

Figure 6. Timeline of patient admission with Multisystem Inflammatory Syndrome in Children (MIS-C) in Geneva and publication of MIS-C case definition and case series. The red stars represent individual MIS-C patients admitted to the hospital. NHSE = National Health Service England; RCPCH = Royal College of Paediatrics and Child Health; CDC = Center for Disease Control and Prevention; WHO = World Health Organization;

These different collaborative MIS-C registries allowed rapid sharing of critical information on the initial presentation, clinical course and response to immunomodulatory treatments of MIS-C patients. In absence of randomized control studies, large retrospectives studies played a significant role not only to increase our knowledge and but also to improve the care of MIS-C patients, by standardizing the diagnostic evaluation and management plan for MIS-C.

This new hyperinflammatory syndrome also represented a unique opportunity to perform a deep dive in the immunological response after an infection with a new virus. Recent translational studies on MIS-C patients using very robust and innovative system-level immune profiling assays were able to identify unique immunological signatures in MIS-C patients that distinguished them from children or adults with acute COVID-19 or children with KD. Furthermore, they also contributed to bring together the different pieces of the immunopathogenesis puzzle of MIS-C including the following possible theories: the persistent exposure of the immune system to SARS-CoV-2 antigen possible via gut translocation, the presence of an autoreactive immune phenotype possibly driven by a superantigenic-like motif of SARS-CoV-2 spike glycoprotein and finally the presence of a

strong autoimmune signature targeting different key organs involved in MIS-C including the gastrointestinal, cardiovascular and neurological systems (Figure 7).

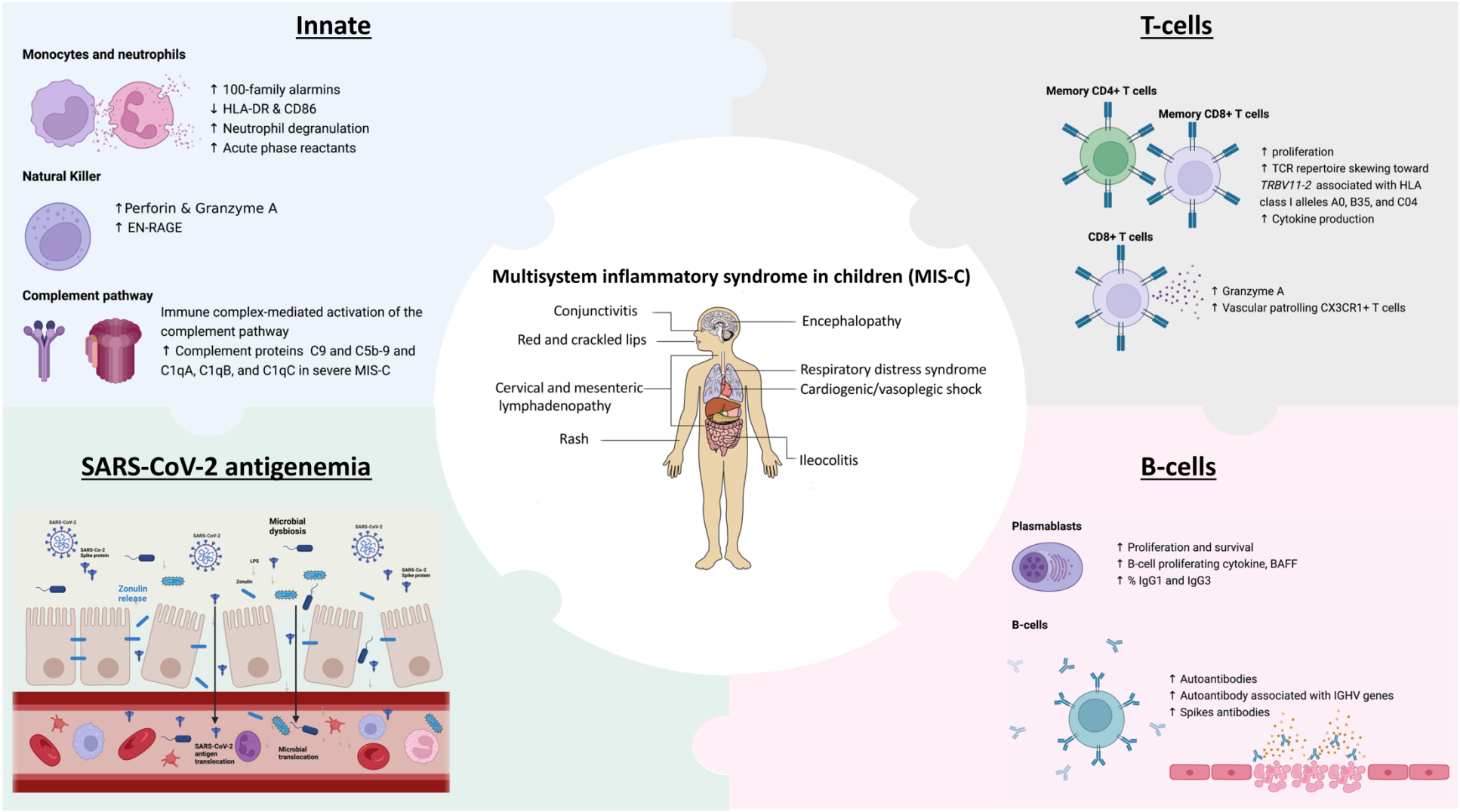

Figure 7. Overview of the innate and adaptative signatures observed in patients with MIS-C and the proposed hypotheses driving the hyperinflammatory response in MIS-C. BAFF= B-cell activating factor; EN-RAGE = extracellular newly identified receptor for advanced glycation end-products binding protein; HLA-DR = major histocompatibility complex II cell surface receptor; IGHV = immunoglobulin heavy variable; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TCR= t-cell receptor; TRBV11-2= t-cell receptor beta variable 11-2; created with BioRender.com and adapted from (110, 139)

Despite the severity of the initial clinical presentation, with numerous MIS-C patients admitted in shock with multiple organ involvement requiring admission to the intensive care unit, most of them responded well to the administration of immunomodulatory treatments and could be discharge home after approximately 1 week of hospitalization. While most of the MIS-C patients recovered from their critical illness, with normalization of the inflammatory and cardiac biomarkers, a significant proportion of them reported persistent or new-onset neuropsychiatric and sleep symptoms at follow-up. This highlights the importance of a multidisciplinary follow-up consultation for children admitted after a critical illness even more so in the presence of a new syndrome for which we lack information on long-term consequences.

Although the incidence and the severity of MIS-C cases appear to decrease over the last months, possibly as a result of both the COVID-19 vaccination campaign and differences in the immunogenic properties of the recent SARS-CoV-2 strains, knowledge gained from the management of MIS-C patients and its extensive immunoprofiling will also be useful for the care of other hyperinflammatory diseases affecting children such as KD or MAS.

References

1. Coronaviridae Study Group of the International Committee on Taxonomy of V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol.* 2020;5(4):536-44.
2. Rothan HA, and Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun.* 2020;109:102433.
3. Organization WH. COVID-19 Weekly epidemiological update-January 17, 2020. https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON229.
4. Organization WH. WHO Director-General's opening remarks at the media briefing on COVID-19. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
5. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell.* 2020;181(2):271-80 e8.
6. Sharma A, and Lal SK. Is tetherin a true antiviral: The influenza a virus controversy. *Rev Med Virol.* 2019;29(3):e2036.
7. Saputri DS, Li S, van Eerden FJ, Rozewicki J, Xu Z, Ismanto HS, et al. Flexible, Functional, and Familiar: Characteristics of SARS-CoV-2 Spike Protein Evolution. *Front Microbiol.* 2020;11:2112.
8. Callaway E. The coronavirus is mutating - does it matter? *Nature.* 2020;585(7824):174-7.
9. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell.* 2020;182(4):812-27 e19.
10. Nicholson TW, Talbot NP, Nickol A, Chadwick AJ, and Lawton O. Respiratory failure and non-invasive respiratory support during the covid-19 pandemic: an update for re-deployed hospital doctors and primary care physicians. *BMJ.* 2020;369:m2446.
11. Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, and Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med.* 2020;8(7):681-6.
12. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med.* 2020;180(7):934-43.
13. Liu Y, Mao B, Liang S, Yang JW, Lu HW, Chai YH, et al. Association between age and clinical characteristics and outcomes of COVID-19. *Eur Respir J.* 2020;55(5).
14. Tian J, Yuan X, Xiao J, Zhong Q, Yang C, Liu B, et al. Clinical characteristics and risk factors associated with COVID-19 disease severity in patients with cancer in Wuhan, China: a multicentre, retrospective, cohort study. *Lancet Oncol.* 2020;21(7):893-903.
15. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* 2020;395(10234):1417-8.

16. Smadja DM, Guerin CL, Chocron R, Yatim N, Boussier J, Gendron N, et al. Angiopoietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients. *Angiogenesis.* 2020;23(4):611-20.
17. Maharaj S, Xue R, and Rojan A. Thrombotic thrombocytopenic purpura (TTP) response following COVID-19 infection: Implications for the ADAMTS-13-von Willebrand factor axis. *J Thromb Haemost.* 2021;19(4):1130-2.
18. Gasecka A, Borovac JA, Guerreiro RA, Giustozzi M, Parker W, Caldeira D, et al. Thrombotic Complications in Patients with COVID-19: Pathophysiological Mechanisms, Diagnosis, and Treatment. *Cardiovasc Drugs Ther.* 2021;35(2):215-29.
19. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, et al. Neurological associations of COVID-19. *Lancet Neurol.* 2020;19(9):767-83.
20. Matschke J, Lutgehetmann M, Hagel C, Sperhake JP, Schroder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 2020;19(11):919-29.
21. Panciani PP, Saraceno G, Zanin L, Renisi G, Signorini L, Battaglia L, et al. SARS-CoV-2: "Three-steps" infection model and CSF diagnostic implication. *Brain Behav Immun.* 2020;87:128-9.
22. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol.* 2020;92(7):699-702.
23. Solomon T. Neurological infection with SARS-CoV-2 - the story so far. *Nat Rev Neurol.* 2021;17(2):65-6.
24. Al-Sarraj S, Troakes C, Hanley B, Osborn M, Richardson MP, Hotopf M, et al. Invited Review: The spectrum of neuropathology in COVID-19. *Neuropathol Appl Neurobiol.* 2021;47(1):3-16.
25. Sinha P, Matthay MA, and Calfee CS. Is a "Cytokine Storm" Relevant to COVID-19? *JAMA Intern Med.* 2020;180(9):1152-4.
26. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
27. Ruan Q, Yang K, Wang W, Jiang L, and Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* 2020;46(5):846-8.
28. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* 2020;130(5):2620-9.
29. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J Med Virol.* 2020;92(7):791-6.
30. Chen L, Liu HG, Liu W, Liu J, Liu K, Shang J, et al. [Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia]. *Zhonghua Jie He He Hu Xi Za Zhi.* 2020;43(0):E005.
31. Sun D, Li H, Lu XX, Xiao H, Ren J, Zhang FR, et al. Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: a single center's observational study. *World J Pediatr.* 2020;16(3):251-9.
32. Cui X, Zhang T, Zheng J, Zhang J, Si P, Xu Y, et al. Children with coronavirus disease 2019: A review of demographic, clinical, laboratory, and imaging features in pediatric patients. *J Med Virol.* 2020;92(9):1501-10.

33. Pathak EB, Salemi JL, Sobers N, Menard J, and Hambleton IR. COVID-19 in Children in the United States: Intensive Care Admissions, Estimated Total Infected, and Projected Numbers of Severe Pediatric Cases in 2020. *J Public Health Manag Pract.* 2020;26(4):325-33.
34. Molloy EJ, and Bearer CF. COVID-19 in children and altered inflammatory responses. *Pediatr Res.* 2020;88(3):340-1.
35. Cui X, Zhao Z, Zhang T, Guo W, Guo W, Zheng J, et al. A systematic review and meta-analysis of children with coronavirus disease 2019 (COVID-19). *J Med Virol.* 2021;93(2):1057-69.
36. Rodriguez-Morales AJ, Cardona-Ospina JA, Gutierrez-Ocampo E, Villamizar-Pena R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. *Travel Med Infect Dis.* 2020;34:101623.
37. Fu L, Wang B, Yuan T, Chen X, Ao Y, Fitzpatrick T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: A systematic review and meta-analysis. *J Infect.* 2020;80(6):656-65.
38. Grant MC, Geoghegan L, Arbyn M, Mohammed Z, McGuinness L, Clarke EL, et al. The prevalence of symptoms in 24,410 adults infected by the novel coronavirus (SARS-CoV-2; COVID-19): A systematic review and meta-analysis of 148 studies from 9 countries. *PLoS One.* 2020;15(6):e0234765.
39. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 Infection in Children. *N Engl J Med.* 2020;382(17):1663-5.
40. Posfay-Barbe KM, Wagner N, Gauthey M, Moussaoui D, Loevy N, Diana A, et al. COVID-19 in Children and the Dynamics of Infection in Families. *Pediatrics.* 2020;146(2).
41. Shekerdemian LS, Mahmood NR, Wolfe KK, Riggs BJ, Ross CE, McKiernan CA, et al. Characteristics and Outcomes of Children With Coronavirus Disease 2019 (COVID-19) Infection Admitted to US and Canadian Pediatric Intensive Care Units. *JAMA Pediatr.* 2020;174(9):868-73.
42. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 Among Children in China. *Pediatrics.* 2020.
43. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, and Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet.* 2020.
44. Dallan C, Romano F, Siebert J, Politi S, Lacroix L, and Sahyoun C. Septic shock presentation in adolescents with COVID-19. *Lancet Child Adolesc Health.* 2020;4(7):e21-e3.
45. Verdoni L, Mazza A, Gervasoni A, Martelli L, Ruggeri M, Ciuffreda M, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet.* 2020;395(10239):1771-8.
46. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, et al. Clinical Characteristics of 58 Children With a Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. *JAMA.* 2020;324(3):259-69.
47. Toubiana J, Poirault C, Corsia A, Bajolle F, Fourgeaud J, Angoulvant F, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ.* 2020;369:m2094.
48. Organization WH. Multisystem inflammatory syndrome in children and adolescents with COVID-19. https://www.who.int/news-room/commentaries/detail/multisystem-

inflammatory-syndrome-in-children-and-adolescents-with-covid-19. Accessed 15.10.2021.
49. Pediatrics AAo. CDC details COVID-19-related inflammatory syndrome in children. https://www.aappublications.org/news/2020/05/14/covid19inflammatory051420. Accessed 11.11.2020.
50. Health RCoPaC. Guidance: paediatric multisystem inflammatory syndrome temporally associated with COVID-19. https://www.rcpch.ac.uk/resources/paediatric-multisystem-inflammatory-syndrome-temporally-associated-covid-19-pims-guidance. Accessed 11.11.2020.
51. Dufort EM, Koumans EH, Chow EJ, Rosenthal EM, Muse A, Rowlands J, et al. Multisystem Inflammatory Syndrome in Children in New York State. *N Engl J Med.* 2020;383(4):347-58.
52. Payne AB, Gilani Z, Godfred-Cato S, Belay ED, Feldstein LR, Patel MM, et al. Incidence of Multisystem Inflammatory Syndrome in Children Among US Persons Infected With SARS-CoV-2. *JAMA Netw Open.* 2021;4(6):e2116420.
53. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF, et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. *N Engl J Med.* 2020;383(4):334-46.
54. Abrams JY, Oster ME, Godfred-Cato SE, Bryant B, Datta SD, Campbell AP, et al. Factors linked to severe outcomes in multisystem inflammatory syndrome in children (MIS-C) in the USA: a retrospective surveillance study. *Lancet Child Adolesc Health.* 2021;5(5):323-31.
55. Feldstein LR, Tenforde MW, Friedman KG, Newhams M, Rose EB, Dapul H, et al. Characteristics and Outcomes of US Children and Adolescents With Multisystem Inflammatory Syndrome in Children (MIS-C) Compared With Severe Acute COVID-19. *JAMA.* 2021;325(11):1074-87.
56. Valverde I, Singh Y, Sanchez-de-Toledo J, Theocharis P, Chikermane A, Di Filippo S, et al. Acute Cardiovascular Manifestations in 286 Children With Multisystem Inflammatory Syndrome Associated With COVID-19 Infection in Europe. *Circulation.* 2021;143(1):21-32.
57. Bermejo IA, Bautista-Rodriguez C, Fraisse A, Voges I, Gatehouse P, Kang H, et al. Short-Term sequelae of Multisystem Inflammatory Syndrome in Children Assessed by CMR. *JACC Cardiovasc Imaging.* 2021;14(8):1666-7.
58. Penner J, Abdel-Mannan O, Grant K, Maillard S, Kucera F, Hassell J, et al. 6-month multidisciplinary follow-up and outcomes of patients with paediatric inflammatory multisystem syndrome (PIMS-TS) at a UK tertiary paediatric hospital: a retrospective cohort study. *Lancet Child Adolesc Health.* 2021;5(7):473-82.
59. Son MBF, Murray N, Friedman K, Young CC, Newhams MM, Feldstein LR, et al. Multisystem Inflammatory Syndrome in Children - Initial Therapy and Outcomes. *N Engl J Med.* 2021;385(1):23-34.
60. Kobayashi R, Dionne A, Ferraro A, Harrild D, Newburger J, VanderPluym C, et al. Detailed Assessment of Left Ventricular Function in Multisystem Inflammatory Syndrome in Children, Using Strain Analysis. *CJC Open.* 2021;3(7):880-7.
61. Matsubara D, Kauffman HL, Wang Y, Calderon-Anyosa R, Nadaraj S, Elias MD, et al. Echocardiographic Findings in Pediatric Multisystem Inflammatory Syndrome Associated With COVID-19 in the United States. *J Am Coll Cardiol.* 2020;76(17):1947-61.

62. Theocharis P, Wong J, Pushparajah K, Mathur SK, Simpson JM, Pascall E, et al. Multimodality cardiac evaluation in children and young adults with multisystem inflammation associated with COVID-19. *Eur Heart J Cardiovasc Imaging.* 2021;22(8):896-903.
63. Blondiaux E, Parisot P, Redheuil A, Tzaroukian L, Levy Y, Sileo C, et al. Cardiac MRI in Children with Multisystem Inflammatory Syndrome Associated with COVID-19. *Radiology.* 2020;297(3):E283-E8.
64. Mannarino S, Raso I, Garbin M, Ghidoni E, Corti C, Goletto S, et al. Cardiac dysfunction in Multisystem Inflammatory Syndrome in Children: An Italian single-center study. *Ital J Pediatr.* 2022;48(1):25.
65. Veraldi N, Vives RR, Blanchard-Rohner G, L'Huillier AG, Wagner N, Rohr M, et al. Endothelial glycocalyx degradation in multisystem inflammatory syndrome in children related to COVID-19. *J Mol Med (Berl).* 2022;100(5):735-46.
66. Alsaied T, Aboulhosn JA, Cotts TB, Daniels CJ, Etheridge SP, Feltes TF, et al. Coronavirus Disease 2019 (COVID-19) Pandemic Implications in Pediatric and Adult Congenital Heart Disease. *J Am Heart Assoc.* 2020;9(12):e017224.
67. Toubiana J, Levy C, Allali S, Jung C, Leruez-Ville M, Varon E, et al. Association between SARS-CoV-2 infection and Kawasaki-like multisystem inflammatory syndrome: a retrospective matched case-control study, Paris, France, April to May 2020. *Euro Surveill.* 2020;25(48).
68. Haghighi Aski B, Manafi Anari A, Abolhasan Choobdar F, Zareh Mahmoudabadi R, and Sakhaei M. Cardiac abnormalities due to multisystem inflammatory syndrome temporally associated with Covid-19 among children: A systematic review and meta-analysis. *Int J Cardiol Heart Vasc.* 2021;33:100764.
69. Wacker J, Malaspinas I, Vallee JP, and Beghetti M. Regression of coronary arteries aneurysms 6 months after multisystem inflammatory syndrome in children (MIS-C). *Eur Heart J.* 2021;42(28):2803.
70. Dionne A, and Newburger JW. The Electrocardiogram in Multisystem Inflammatory Syndrome in Children: Mind Your Ps and Qs. *J Pediatr.* 2021;234:10-1.
71. Duarte-Neto AN, Caldini EG, Gomes-Gouvea MS, Kanamura CT, de Almeida Monteiro RA, Ferranti JF, et al. An autopsy study of the spectrum of severe COVID-19 in children: From SARS to different phenotypes of MIS-C. *EClinicalMedicine.* 2021;35:100850.
72. Dolhnikoff M, Ferreira Ferranti J, de Almeida Monteiro RA, Duarte-Neto AN, Soares Gomes-Gouvea M, Viu Degaspare N, et al. SARS-CoV-2 in cardiac tissue of a child with COVID-19-related multisystem inflammatory syndrome. *Lancet Child Adolesc Health.* 2020;4(10):790-4.
73. Yang X, Chang Y, and Wei W. Endothelial Dysfunction and Inflammation: Immunity in Rheumatoid Arthritis. *Mediators Inflamm.* 2016;2016:6813016.
74. Zhang J, Tecson KM, and McCullough PA. Endothelial dysfunction contributes to COVID-19-associated vascular inflammation and coagulopathy. *Rev Cardiovasc Med.* 2020;21(3):315-9.
75. Johansson PI, Henriksen HH, Stensballe J, Gybel-Brask M, Cardenas JC, Baer LA, et al. Traumatic Endotheliopathy: A Prospective Observational Study of 424 Severely Injured Patients. *Ann Surg.* 2017;265(3):597-603.

76. Ostrowski SR, Haase N, Muller RB, Moller MH, Pott FC, Perner A, et al. Association between biomarkers of endothelial injury and hypocoagulability in patients with severe sepsis: a prospective study. *Crit Care.* 2015;19:191.
77. Smart L, Macdonald SPJ, Burrows S, Bosio E, Arendts G, and Fatovich DM. Endothelial glycocalyx biomarkers increase in patients with infection during Emergency Department treatment. *J Crit Care.* 2017;42:304-9.
78. Buijsers B, Yanginlar C, de Nooijer A, Grondman I, Maciej-Hulme ML, Jonkman I, et al. Increased Plasma Heparanase Activity in COVID-19 Patients. *Front Immunol.* 2020;11:575047.
79. Fraser DD, Patterson EK, Slessarev M, Gill SE, Martin C, Daley M, et al. Endothelial Injury and Glycocalyx Degradation in Critically Ill Coronavirus Disease 2019 Patients: Implications for Microvascular Platelet Aggregation. *Crit Care Explor.* 2020;2(9):e0194.
80. Ciftel M, Ates N, and Yilmaz O. Investigation of endothelial dysfunction and arterial stiffness in multisystem inflammatory syndrome in children. *Eur J Pediatr.* 2022;181(1):91-7.
81. Ankola AA, Bradford VR, Newburger JW, Emani S, Dionne A, Friedman K, et al. Coagulation profiles and viscoelastic testing in multisystem inflammatory syndrome in children. *Pediatr Blood Cancer.* 2021;68(12):e29355.
82. Al-Ghafry M, Vagrecha A, Malik M, Levine C, Uster E, Aygun B, et al. Multisystem inflammatory syndrome in children (MIS-C) and the prothrombotic state: Coagulation profiles and rotational thromboelastometry in a MIS-C cohort. *J Thromb Haemost.* 2021;19(7):1764-70.
83. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost.* 2020;18(7):1738-42.
84. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol.* 2020;7(8):e575-e82.
85. Aronoff SC, Hall A, and Del Vecchio MT. The Natural History of Severe Acute Respiratory Syndrome Coronavirus 2-Related Multisystem Inflammatory Syndrome in Children: A Systematic Review. *J Pediatric Infect Dis Soc.* 2020;9(6):746-51.
86. Whitworth H, Sartain SE, Kumar R, Armstrong K, Ballester L, Betensky M, et al. Rate of thrombosis in children and adolescents hospitalized with COVID-19 or MIS-C. *Blood.* 2021;138(2):190-8.
87. Bilaloglu S, Aphinyanaphongs Y, Jones S, Iturrate E, Hochman J, and Berger JS. Thrombosis in Hospitalized Patients With COVID-19 in a New York City Health System. *JAMA.* 2020;324(8):799-801.
88. Hospital JHACs. https://ClinicalTrials.gov/show/NCT04354155; 2020.
89. Goldenberg NA, Sochet A, Albisetti M, Biss T, Bonduel M, Jaffray J, et al. Consensus-based clinical recommendations and research priorities for anticoagulant thromboprophylaxis in children hospitalized for COVID-19-related illness. *J Thromb Haemost.* 2020;18(11):3099-105.
90. Miller J, Cantor A, Zachariah P, Ahn D, Martinez M, and Margolis KG. Gastrointestinal Symptoms as a Major Presentation Component of a Novel Multisystem Inflammatory

Syndrome in Children That Is Related to Coronavirus Disease 2019: A Single Center Experience of 44 Cases. *Gastroenterology.* 2020;159(4):1571-4 e2.
91. Calinescu AM, Vidal I, Grazioli S, Lacroix L, and Wildhaber BE. Beware of Too Aggressive Approach in Children With Acute Abdomen During COVID-19 Outbreak! *Ann Surg.* 2020;272(3):e244-e5.
92. Sahn B, Eze OP, Edelman MC, Chougar CE, Thomas RM, Schleien CL, et al. Features of Intestinal Disease Associated With COVID-Related Multisystem Inflammatory Syndrome in Children. *J Pediatr Gastroenterol Nutr.* 2021;72(3):384-7.
93. Yonker LM, Gilboa T, Ogata AF, Senussi Y, Lazarovits R, Boribong BP, et al. Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier. *J Clin Invest.* 2021;131(14).
94. Sa M, Mirza L, Carter M, Carlton Jones L, Gowda V, Handforth J, et al. Systemic Inflammation Is Associated With Neurologic Involvement in Pediatric Inflammatory Multisystem Syndrome Associated With SARS-CoV-2. *Neurol Neuroimmunol Neuroinflamm.* 2021;8(4).
95. Abdel-Mannan O, Eyre M, Lobel U, Bamford A, Eltze C, Hameed B, et al. Neurologic and Radiographic Findings Associated With COVID-19 Infection in Children. *JAMA Neurol.* 2020;77(11):1440-5.
96. LaRovere KL, Riggs BJ, Poussaint TY, Young CC, Newhams MM, Maamari M, et al. Neurologic Involvement in Children and Adolescents Hospitalized in the United States for COVID-19 or Multisystem Inflammatory Syndrome. *JAMA Neurol.* 2021;78(5):536-47.
97. Ray STJ, Abdel-Mannan O, Sa M, Fuller C, Wood GK, Pysden K, et al. Neurological manifestations of SARS-CoV-2 infection in hospitalised children and adolescents in the UK: a prospective national cohort study. *Lancet Child Adolesc Health.* 2021;5(9):631-41.
98. Lindan CE, Mankad K, Ram D, Kociolek LK, Silvera VM, Boddaert N, et al. Neuroimaging manifestations in children with SARS-CoV-2 infection: a multinational, multicentre collaborative study. *Lancet Child Adolesc Health.* 2021;5(3):167-77.
99. Schupper AJ, Yaeger KA, and Morgenstern PF. Neurological manifestations of pediatric multi-system inflammatory syndrome potentially associated with COVID-19. *Childs Nerv Syst.* 2020;36(8):1579-80.
100. Abel D, Shen MY, Abid Z, Hennigan C, Boneparth A, Miller EH, et al. Encephalopathy and bilateral thalamic lesions in a child with MIS-C associated with COVID-19. *Neurology.* 2020;95(16):745-8.
101. Sato T, Ushiroda Y, Oyama T, Nakatomi A, Motomura H, and Moriuchi H. Kawasaki disease-associated MERS: pathological insights from SPECT findings. *Brain Dev.* 2012;34(7):605-8.
102. Takanashi J, Shirai K, Sugawara Y, Okamoto Y, Obonai T, and Terada H. Kawasaki disease complicated by mild encephalopathy with a reversible splenial lesion (MERS). *J Neurol Sci.* 2012;315(1-2):167-9.
103. Vanderschueren G, Schotsmans K, Marechal E, and Crols R. Mild encephalitis with reversible splenial (MERS) lesion syndrome due to influenza B virus. *Pract Neurol.* 2018;18(5):391-2.
104. Feraco P, Porretti G, Marchio G, Bellizzi M, and Recla M. Mild Encephalitis/Encephalopathy with Reversible Splenial Lesion (MERS) due to

Cytomegalovirus: Case Report and Review of the Literature. *Neuropediatrics.* 2018;49(1):68-71.
105. Soon GS, Rodan LH, Laughlin S, Laxer RM, Benseler S, and Silverman ED. Reversible splenial lesion syndrome in pediatric systemic lupus erythematosus. *J Rheumatol.* 2012;39(8):1698-9.
106. Tada H, Takanashi J, Barkovich AJ, Oba H, Maeda M, Tsukahara H, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion. *Neurology.* 2004;63(10):1854-8.
107. Frontera J, Mainali S, Fink EL, Robertson CL, Schober M, Ziai W, et al. Global Consortium Study of Neurological Dysfunction in COVID-19 (GCS-NeuroCOVID): Study Design and Rationale. *Neurocrit Care.* 2020;33(1):25-34.
108. Cheung EW, Zachariah P, Gorelik M, Boneparth A, Kernie SG, Orange JS, et al. Multisystem Inflammatory Syndrome Related to COVID-19 in Previously Healthy Children and Adolescents in New York City. *JAMA.* 2020;324(3):294-6.
109. Chiotos K, Bassiri H, Behrens EM, Blatz AM, Chang J, Diorio C, et al. Multisystem Inflammatory Syndrome in Children During the Coronavirus 2019 Pandemic: A Case Series. *J Pediatric Infect Dis Soc.* 2020;9(3):393-8.
110. Belhadjer Z, Meot M, Bajolle F, Khraiche D, Legendre A, Abakka S, et al. Acute heart failure in multisystem inflammatory syndrome in children (MIS-C) in the context of global SARS-CoV-2 pandemic. *Circulation.* 2020.
111. Deza Leon MP, Redzepi A, McGrath E, Abdel-Haq N, Shawaqfeh A, Sethuraman U, et al. COVID-19-Associated Pediatric Multisystem Inflammatory Syndrome. *J Pediatric Infect Dis Soc.* 2020;9(3):407-8.
112. Pouletty M, Borocco C, Ouldali N, Caseris M, Basmaci R, Lachaume N, et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis.* 2020;79(8):999-1006.
113. Lee PY, Day-Lewis M, Henderson LA, Friedman KG, Lo J, Roberts JE, et al. Distinct clinical and immunological features of SARS-CoV-2-induced multisystem inflammatory syndrome in children. *J Clin Invest.* 2020;130(11):5942-50.
114. Henderson LA, Canna SW, Friedman KG, Gorelik M, Lapidus SK, Bassiri H, et al. 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. *Arthritis Rheumatol.* 2022;74(4):e1-e20.
115. Schlapbach LJ, Andre MC, Grazioli S, Schobi N, Ritz N, Aebi C, et al. Best Practice Recommendations for the Diagnosis and Management of Children With Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2 (PIMS-TS; Multisystem Inflammatory Syndrome in Children, MIS-C) in Switzerland. *Front Pediatr.* 2021;9:667507.
116. Ouldali N, Toubiana J, Antona D, Javouhey E, Madhi F, Lorrot M, et al. Association of Intravenous Immunoglobulins Plus Methylprednisolone vs Immunoglobulins Alone With Course of Fever in Multisystem Inflammatory Syndrome in Children. *JAMA.* 2021.
117. McArdle AJ, Vito O, Patel H, Seaby EG, Shah P, Wilson C, et al. Treatment of Multisystem Inflammatory Syndrome in Children. *N Engl J Med.* 2021;385(1):11-22.
118. Rauniyar R, Mishra A, Kharel S, Giri S, Rauniyar R, Yadav S, et al. IVIG plus Glucocorticoids versus IVIG Alone in Multisystem Inflammatory Syndrome in Children

(MIS-C) Associated with COVID-19: A Systematic Review and Meta-Analysis. *Can J Infect Dis Med Microbiol.* 2022;2022:9458653.
119. Cole LD, Osborne CM, Silveira LJ, Rao S, Lockwood JM, Kunkel MJ, et al. IVIG Compared to IVIG Plus Infliximab in Multisystem Inflammatory Syndrome in Children. *Pediatrics.* 2021.
120. Belhadjer Z, Auriau J, Meot M, Oualha M, Renolleau S, Houyel L, et al. Addition of Corticosteroids to Immunoglobulins Is Associated With Recovery of Cardiac Function in Multi-Inflammatory Syndrome in Children. *Circulation.* 2020;142(23):2282-4.
121. Capone CA, Subramony A, Sweberg T, Schneider J, Shah S, Rubin L, et al. Characteristics, Cardiac Involvement, and Outcomes of Multisystem Inflammatory Syndrome of Childhood Associated with severe acute respiratory syndrome coronavirus 2 Infection. *J Pediatr.* 2020;224:141-5.
122. Kaushik S, Aydin SI, Derespina KR, Bansal PB, Kowalsky S, Trachtman R, et al. Multisystem Inflammatory Syndrome in Children Associated with Severe Acute Respiratory Syndrome Coronavirus 2 Infection (MIS-C): A Multi-institutional Study from New York City. *J Pediatr.* 2020;224:24-9.
123. Fisher CJ, Jr., Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA.* 1994;271(23):1836-43.
124. Celikel E, Tekin ZE, Aydin F, Emeksiz S, Uyar E, Ozcan S, et al. Role of Biological Agents in the Treatment of SARS-CoV-2-Associated Multisystem Inflammatory Syndrome in Children. *J Clin Rheumatol.* 2022;28(2):e381-e7.
125. Mathieu E, Ritchie H, Ortiz-Ospina E, Roser M, Hasell J, Appel C, et al. A global database of COVID-19 vaccinations. *Nat Hum Behav.* 2021;5(7):947-53.
126. Levy M, Recher M, Hubert H, Javouhey E, Flechelles O, Leteurtre S, et al. Multisystem Inflammatory Syndrome in Children by COVID-19 Vaccination Status of Adolescents in France. *JAMA.* 2022;327(3):281-3.
127. Zambrano LD, Newhams MM, Olson SM, Halasa NB, Price AM, Boom JA, et al. Effectiveness of BNT162b2 (Pfizer-BioNTech) mRNA Vaccination Against Multisystem Inflammatory Syndrome in Children Among Persons Aged 12-18 Years - United States, July-December 2021. *MMWR Morb Mortal Wkly Rep.* 2022;71(2):52-8.
128. Karatzios C, Scuccimarri R, Chedeville G, Basfar W, Bullard J, and Stein DR. Multisystem Inflammatory Syndrome Following SARS-CoV-2 Vaccination in Two Children. *Pediatrics.* 2022.
129. Yousaf AR, Cortese MM, Taylor AW, Broder KR, Oster ME, Wong JM, et al. Reported cases of multisystem inflammatory syndrome in children aged 12-20 years in the USA who received a COVID-19 vaccine, December, 2020, through August, 2021: a surveillance investigation. *Lancet Child Adolesc Health.* 2022;6(5):303-12.
130. Syrimi E, Fennell E, Richter A, Vrljicak P, Stark R, Ott S, et al. The immune landscape of SARS-CoV-2-associated Multisystem Inflammatory Syndrome in Children (MIS-C) from acute disease to recovery. *iScience.* 2021;24(11):103215.
131. Pierce CA, Preston-Hurlburt P, Dai Y, Aschner CB, Cheshenko N, Galen B, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Sci Transl Med.* 2020;12(564).

132. Carter MJ, Fish M, Jennings A, Doores KJ, Wellman P, Seow J, et al. Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. *Nat Med.* 2020;26(11):1701-7.
133. Sacco K, Castagnoli R, Vakkilainen S, Liu C, Delmonte OM, Oguz C, et al. Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19. *Nat Med.* 2022;28(5):1050-62.
134. Gruber C, Patel R, Trachman R, Lepow L, Amanat F, Krammer F, et al. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). *medRxiv.* 2020.
135. Porritt RA, Binek A, Paschold L, Rivas MN, McArdle A, Yonker LM, et al. The autoimmune signature of hyperinflammatory multisystem inflammatory syndrome in children. *J Clin Invest.* 2021;131(20).
136. Rapp M, Wintergerst MWM, Kunz WG, Vetter VK, Knott MML, Lisowski D, et al. CCL22 controls immunity by promoting regulatory T cell communication with dendritic cells in lymph nodes. *J Exp Med.* 2019;216(5):1170-81.
137. Yamashita U, and Kuroda E. Regulation of macrophage-derived chemokine (MDC, CCL22) production. *Crit Rev Immunol.* 2002;22(2):105-14.
138. Rodriguez-Smith JJ, Verweyen EL, Clay GM, Esteban YM, de Loizaga SR, Baker EJ, et al. Inflammatory biomarkers in COVID-19-associated multisystem inflammatory syndrome in children, Kawasaki disease, and macrophage activation syndrome: a cohort study. *Lancet Rheumatol.* 2021;3(8):e574-e84.
139. Ramaswamy A, Brodsky NN, Sumida TS, Comi M, Asashima H, Hoehn KB, et al. Immune dysregulation and autoreactivity correlate with disease severity in SARS-CoV-2-associated multisystem inflammatory syndrome in children. *Immunity.* 2021;54(5):1083-95 e7.
140. Vella LA, Giles JR, Baxter AE, Oldridge DA, Diorio C, Kuri-Cervantes L, et al. Deep immune profiling of MIS-C demonstrates marked but transient immune activation compared to adult and pediatric COVID-19. *Sci Immunol.* 2021;6(57).
141. Porritt RA, Paschold L, Rivas MN, Cheng MH, Yonker LM, Chandnani H, et al. HLA class I-associated expansion of TRBV11-2 T cells in multisystem inflammatory syndrome in children. *J Clin Invest.* 2021;131(10).
142. Cheng MH, Zhang S, Porritt RA, Noval Rivas M, Paschold L, Willscher E, et al. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. *Proc Natl Acad Sci U S A.* 2020;117(41):25254-62.
143. Anderson EM, Diorio C, Goodwin EC, McNerney KO, Weirick ME, Gouma S, et al. SARS-CoV-2 antibody responses in children with MIS-C and mild and severe COVID-19. *medRxiv.* 2020.
144. Rostad CA, Chahroudi A, Mantus G, Lapp SA, Teherani M, Macoy L, et al. Quantitative SARS-CoV-2 Serology in Children With Multisystem Inflammatory Syndrome (MIS-C). *Pediatrics.* 2020;146(6).
145. Bartsch YC, Wang C, Zohar T, Fischinger S, Atyeo C, Burke JS, et al. Humoral signatures of protective and pathological SARS-CoV-2 infection in children. *Nat Med.* 2021;27(3):454-62.
146. Consiglio CR, Cotugno N, Sardh F, Pou C, Amodio D, Rodriguez L, et al. The Immunology of Multisystem Inflammatory Syndrome in Children with COVID-19. *Cell.* 2020;183(4):968-81 e7.

147. Galeotti C, Kaveri SV, and Bayry J. IVIG-mediated effector functions in autoimmune and inflammatory diseases. *Int Immunol.* 2017;29(11):491-8.
148. Yonker LM, Shen K, and Kinane TB. Lessons unfolding from pediatric cases of COVID-19 disease caused by SARS-CoV-2 infection. *Pediatr Pulmonol.* 2020;55(5):1085-6.
149. Grazioli S, Tavaglione F, Torriani G, Wagner N, Rohr M, L'Huillier AG, et al. Immunological assessment of pediatric multisystem inflammatory syndrome related to COVID-19. *J Pediatric Infect Dis Soc.* 2020.
150. Farooqi KM, Chan A, Weller RJ, Mi J, Jiang P, Abrahams E, et al. Longitudinal Outcomes for Multisystem Inflammatory Syndrome in Children. *Pediatrics.* 2021;148(2).
151. Davies P, du Pre P, Lillie J, and Kanthimathinathan HK. One-Year Outcomes of Critical Care Patients Post-COVID-19 Multisystem Inflammatory Syndrome in Children. *JAMA Pediatr.* 2021;175(12):1281-3.
152. Enner S, Shah YD, Ali A, Cerise JE, Esposito J, Rubin L, et al. Patients Diagnosed with Multisystem Inflammatory Syndrome in Children Have Persistent Neurologic, Sleep, and Psychiatric Symptoms After Hospitalization. *J Child Neurol.* 2022;37(5):426-33.
153. Capone CA, Misra N, Ganigara M, Epstein S, Rajan S, Acharya SS, et al. Six Month Follow-up of Patients With Multi-System Inflammatory Syndrome in Children. *Pediatrics.* 2021;148(4).
154. Schofield-Robinson OJ, Lewis SR, Smith AF, McPeake J, and Alderson P. Follow-up services for improving long-term outcomes in intensive care unit (ICU) survivors. *Cochrane Database Syst Rev.* 2018;11:CD012701.