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Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2

Caroline Diorio, … , David T. Teachey, Hamid Bassiri

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Background: Initial reports from the Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) pandemic described children as being less susceptible to Coronavirus Disease 2019 (COVID-19) than adults. Subsequently, a severe and novel pediatric disorder termed Multisystem Inflammatory Syndrome in Children (MIS-C) emerged. We report on unique hematologic and immunologic parameters that distinguish between COVID-19 and MIS-C and provide insight into pathophysiology.

Methods: We prospectively enrolled hospitalized patients with evidence of SARS-CoV-2 infection and classified them as having MIS-C or COVID-19. Patients with COVID-19 were classified as having either minimal or severe disease. Cytokine profiles, viral cycle thresholds (Cts), blood smears, and soluble C5b-9 values were analyzed with clinical data. Twenty patients were enrolled (9 severe COVID-19, 5 minimal COVID-19, and 6 MIS-C). Five cytokines (IFN-γ, IL-10, IL-6, IL-8 and TNF-α) contributed to the analysis. TNF-α and IL-10 discriminated between patients with MIS-C and severe COVID-19. Cts and burr cells on blood smears also differentiated between patients with severe COVID-19 and those with MIS-C.

Conclusion: Pediatric patients with SARS-CoV-2 are at risk for critical illness with severe COVID-19 and MIS-C. Cytokine profiling and examination of peripheral blood smears may distinguish between patients with […]

Multisystem Inflammatory Syndrome in Children and COVID-19 are distinct presentations of SARS-CoV-2

Caroline Diorio, MD, [1,2] Sarah E. Henrickson, MD, PhD, [1,3] Laura A. Vella, MD, PhD, [1,4] Kevin O. McNerney MD, MSTR, [1,2] Julie Chase MD, PhD,[1,5] Chakkapong Burudpakdee, BS, [1] Jessica H. Lee, BS, [1] Cristina Jasen, BA, [1,3] Fran Balamuth, MD, PhD, MSCE,[6] David M. Barrett, MD, PhD, [1,2] Brenda L. Banwell, MD,[1,7] Kathrin M. Bernt, MD,[2] Allison M. Blatz, MD,[4] Kathleen Chiotos, MD, MSCE,[8] Brian T. Fisher DO, MPH, MSCE,[1,4] Julie C. Fitzgerald, MD, PhD, MSCE,[8] Jeffrey S. Gerber MD, PhD,[4] Kandace Gollomp, MD,[1,9] Christopher Gray, MS, LCGC,[1] Stephan A. Grupp, MD, PhD,[2] Rebecca M. Harris, MD,[4] Todd J. Kilbaugh, MD,[8] Audrey R. Odom John MD, PhD,[4] Michele Lambert, MD, MSTR,[1,9] Emily J. Liebling, MD,[5] Michele E. Paessler, DO,[1,10] Whitney Petrosa, CPNP-AC,[1] Charles Phillips, MD, MSHP,[2] Anne F. Reilly, MD, MPH,[1,2] Neil D. Romberg, MD,[1,3] Alix Seif, MD, MPH,[1,2] Deborah A. Sesok-Pizzini, MD, MBA,[10] Kathleen E. Sullivan MD, PhD,[1,3] Julie Vardaro, MHA, PMP, Edward M. Behrens, MD,*[1,5] David T. Teachey, MD,* [1,2] Hamid Bassiri, MD, PhD*[1,4]

*These authors contributed equally to this work.

[1]Immune Dysregulation Frontier Program, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[2]Division of Oncology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[3]Division of Allergy and Immunology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[4]Division of Infectious Diseases, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[5]Division of Rheumatology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[6]Division of Emergency Medicine, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[7]Division of Neurology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

[8]Division of Critical Care Medicine, Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA
[9]Division of Hematology, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA
[10]Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

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Corresponding Author & Address for Reprints:

Edward M. Behrens
Associate Professor of Pediatrics
The Children's Hospital of Philadelphia
University of Pennsylvania Perelman School of Medicine
Room 1107A ARC
3615 Civic Centre Blvd
Philadelphia, PA 19107
Phone: 215-590-7180 Fax: 215-590-1258
Email: behrens@email.chop.edu

CONFLICTS OF INTEREST

DTT serves on advisory boards for Janssen, Amgen, La Roche, Sobi, and Humanigen. HB has stock ownership in CSL Behring. SH serves on the advisory board for Horizon Pharma. AOJ serves on the advisory board of Pluton Biosciences. MPL is an advisory board member for Octapharma and Shionogi, a consultant for Amgen, Novartis, Shionogi, Dova, Bayer, and has received research funding from Sysmex, Novartis, and Astra Zeneca. KES received personal fees from Elsevier and Immune Deficiency Foundation.

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ABSTRACT

Background: Initial reports from the Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) pandemic described children as being less susceptible to Coronavirus Disease 2019 (COVID-19) than adults. Subsequently, a severe and novel pediatric disorder termed Multisystem Inflammatory Syndrome in Children (MIS-C) emerged. We report on unique hematologic and immunologic parameters that distinguish between COVID-19 and MIS-C and provide insight into pathophysiology.

Methods: We prospectively enrolled hospitalized patients with evidence of SARS-CoV-2 infection and classified them as having MIS-C or COVID-19. Patients with COVID-19 were classified as having either minimal or severe disease. Cytokine profiles, viral cycle thresholds (Cts), blood smears, and soluble C5b-9 values were analyzed with clinical data.

Results: Twenty patients were enrolled (9 severe COVID-19, 5 minimal COVID-19, and 6 MIS-C). Five cytokines (IFN-γ, IL-10, IL-6, IL-8 and TNF-α) contributed to the analysis. TNF-α and IL-10 discriminated between patients with MIS-C and severe COVID-19. The presence of burr cells on blood smears, as well as Cts, differentiated between patients with severe COVID-19 and those with MIS-C.

Conclusions: Pediatric patients with SARS-CoV-2 are at risk for critical illness with severe COVID-19 and MIS-C. Cytokine profiling and examination of peripheral blood smears may distinguish between patients with MIS-C and severe COVID-19.

SUMMARY

MIS-C and COVID-19 are different manifestation of SARS-CoV-2 in pediatric patients and may be distinguished by cytokine panels and peripheral blood smears.

INTRODUCTION

Initial reports from the Coronavirus Disease 2019 (COVID-19) pandemic indicated that children were less severely affected by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection than adults.(1-3) The largest retrospective case series of 2135 children from China reported that <5% of children affected with SARS-CoV-2 had severe disease with only one pediatric death. (2)

On April 27th, 2020 the National Health Service (NHS) in the United Kingdom (UK) issued an alert about pediatric patients presenting with a severe "Kawasaki-like" syndrome in the setting of recently proven or suspected SARS-CoV-2 infection, termed "pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2" (PIMS-TS).(4, 5) These patients presented with disease in the absence of preceding respiratory distress. A similar hyperinflammatory syndrome with features of Kawasaki disease (KD) was reported in Italy.(6)

On May 8th, 2020 the Centers for Disease Control (CDC) in the United States issued an alert describing a new entity termed Multisystem Inflammatory Syndrome in Children (MIS-C) associated with COVID-19.(7) MIS-C is defined by clinically severe illness requiring hospitalization with fever, inflammatory marker elevation and multisystem organ dysfunction in the setting of recent proven or probable SARS-CoV-2 infection, and in the absence of an alternative likely explanation.(7)

KD is a medium vessel vasculitis that occurs almost exclusively in infants and children.(8) It has a seasonal variation, implying an infectious trigger, and has been associated with viruses, including other coronaviruses.(9-11) The clinical diagnosis is defined by fevers for five days and at least four of five cardinal exam findings (bilateral conjunctival injection, mucosal changes, peripheral extremity changes, polymorphous rash and cervical lymphadenopathy).(12) Patients with at least five days of fever with fewer physical exam findings who meet laboratory criteria

may be diagnosed with incomplete KD.(8) If untreated, KD is associated with the development of coronary artery dilatation and aneurysms in approximately 20% of patients.(8) Shock or vascular compromise can occur, but are rare, affecting less than 10% of patients.(13) In contrast, the majority of patients with MIS-C reported to date have presented with shock requiring inotropic support.(5, 6)

Initial reports of MIS-C are restricted to retrospective clinical case series. Moreover, they have not compared clinical or biologic features of MIS-C with other presentations of SARS-CoV-2. It is unknown if the processes that mediate MIS-C are similar to the processes that lead to life-threatening respiratory failure and shock with COVID-19. The reports of severe acute illness after SARS-CoV-2 in children are scant. Based on the clinical phenotype, we hypothesized that children with MIS-C are presenting with a syndrome that has not been previously described, distinct from both KD and severe COVID-19 infection. We utilized an extant multidisciplinary Dysregulated Immunity team to perform a systematic, comprehensive, prospective evaluation of clinical and laboratory biomarkers in children who presented with minimal COVID-19, severe COVID-19, and MIS-C. This evaluation included cytokine profiles, measures of viral burden and markers of vascular and endothelial damage. This is a prospective report comparing life-threatening complications of SARS-CoV-2 in children and provides information on disease biology as well as clinical information that can help practitioners distinguish between the clinical syndromes.

RESULTS

Between April 3, 2020 and May 15, 2020, 26 patients were enrolled. Twenty patients, on whom complete correlative data were available, were included in the final cohort (Supplemental Figure

S1). Demographics and narrative summaries of the MIS-C, severe COVID-19 and minimal COVID-19 cohorts are presented in Table 1 and Table 2. Some clinical details of three patients in the MIS-C cohort have been reported in a previous case series.(14)

Patients in both the MIS-C and severe COVID-19 cohorts had grossly abnormal laboratory values (Supplemental Table S1). Patients in the severe COVID-19 cohort had several clinical features associated with both macrophage activation syndrome (MAS) and shock, such as highly elevated ferritins, elevated transaminases, elevated lactate, thrombocytopenia and prolonged partial thromboplastin times (PTT). Patients in the MIS-C cohort had findings associated with vascular injury and cardiac disease, as evidenced by highly elevated D-dimer and B-type natriuretic protein levels.

The MIS-C cohort patients were younger than the severe or minimal COVID-19 patients (median age 6 versus 16 and 13 years, respectively). The majority of patients in the MIS-C and severe COVID-19 cohorts had lymphopaenia during their hospital admission; only 2 patients in the minimal cohort had lymphopaenia. Of note, 4 out of 5 patients in the severe COVID-19 cohort had a co-infection with an additional organism compared to only 1 out of 6 in the MIS-C cohort. There were no statistically significant differences in race or ethnic background between patients in each group; there was a higher rate of acute respiratory distress syndrome (ARDS) amongst black children (N=4) than children of other races (N=2; 1 other, 1 declined, 0 white; $p = 0.044$, Chi-square test). All children who developed ARDS had an existing comorbidity.

Two patients in the MIS-C cohort met classical criteria for KD and two met criteria for incomplete KD (Supplemental Tables S2 and S3). Five patients were treated with vasoactive infusions, four patients had evidence of myocardial dysfunction and one had evidence of

coronary artery dilatation. All patients were treated with intravenous immune globulin (IVIG) and intravenous methylprednisone.

We sought to identify plasma cytokine patterns that reliably differentiated between MIS-C and severe COVID-19. Graphs of all cytokines are presented in Supplemental Figure S2. Five cytokines (IL-1β, IL-2, IL-4, IL-12p70 and IL-13) were excluded from further analyses because they were generally not elevated in patients. Several patients across all three cohorts had high IFN-γ. Importantly, this demonstrates that the hypercytokinemia observed in our patients reflects a specific hyperinflammatory process, and that significant elevations in IL-1β, IL-2, IL-4, IL-12p70 or IL-13may indicate the presence of a different disease process.

Heatmaps of all patients separated based on the presence or absence of co-infection are presented in Figure 1A. In order to determine if cytokines could robustly classify the different phenotypes, subsequent analyses included all patients regardless of their co-infection status. We next considered the cytokine profile as a 5-dimensional vector, hypothesizing that either the direction or the magnitude of each vector may predict clinical cohort; neither were statistically significant (Figure 1B&C). Based on exploratory multi-variable logistic regression modelling, the sum of IL-10 and TNF-α levels was hypothesized to differentiate between MIS-C and severe non-MIS-C patients. The sum of IL-10 and TNF-α levels uniquely identified MIS-C from severe COVID-19 presentations (Figure 1D; mean [95% CI]; Severe: 30.06 [9.54-50.6] versus MIS-C: 82.25 [32.5-132.0], p=0.036).

We hypothesized that MIS-C, as a putative post-infectious syndrome, should be associated with lower viral burden, when compared to severe COVID-19. SARS-CoV-2 RT-PCR cycle threshold (Cts) from nasopharyngeal aspirates (which inversely correlate with viral burden) were predictive of the clinical cohorts. Severe COVID-19 patients presented with low Cts while those

with MIS-C presented with high Cts (mean [95% CI]; Severe: 28.0 [26.8–29.1] versus MIS-C: 37.9 [34.8–41.0], p=0.011; Figure 2). Univariate linear regression against plasma cytokine concentrations revealed a correlation with IL-10 levels (p = 0.02). In four patients, repeat cytokine panels were drawn to assess clinical response to immunomodulatory therapy (supplemental Figure S3).

We postulated that immune activation related to SARS-CoV-2 was associated with endothelial dysfunction, and we measured soluble (sC5b-9) as a candidate biomarker. sC5b-9 represents the activation product of the terminal complement cascade, and elevations of this marker have been associated with microangiopathy in a number of settings.(15, 16) Figure 3A demonstrates that sC5b-9 was significantly elevated in patients with severe COVID-19 relative to those with minimal COVID-19 (mean [95% CI]; Minimal: 186.4 [91.16-281.6] versus Severe: 555.0 [285.9–814.1], p=0.046). While sC5b-9 values also trended higher for MIS-C (mean [95% CI]; 380.5 [97.7–663.3]), this did not reach statistical significance. Univariate linear regression modelling revealed that sC5b-9 concentrations correlated in a statistically significant manner with IL-6, IL-8, and TNF-α (Figure 3B). These data are consistent with previous reports of sC5b-9 being associated with endothelial damage and release of neutrophil activating IL-8.(17) Peripheral blood smears were available on 13 patients (3 minimal COVID-19, 5 severe COVID-19 and 5 MIS-C). Consistent with microangiopathy, examination revealed at least rare schistocytes in 67% of minimal COVID-19, 80% of severe COVID-19, and 100% of MIS-C patients (Figure 3C&D). We note that 2 of the 3 minimal COVID-19 patients had sickle cell disease as a comorbidity which may confound the interpretation of schistocytes in this group. (18) Burr cells were absent in patients with minimal disease but present in 40% of patients with

severe disease. All patients with MIS-C had at least some burr cells present, with 60% of patients having 4+ burr cells (Figure 3C & 3D).

Patients with MIS-C were noted to have a marked increase in their white blood cell counts; longitudinal measures of absolute neutrophil count (ANC) and absolute lymphocyte count (ALC) for all severe COVID-19 and MIS-C patients are shown in Supplemental Figure S4A. Many of the MIS-C patients were noted to have increased numbers of immature myeloid cells as well (data not shown). Consistent with this myeloid activation, we noted a remarkable number of neutrophils with toxic granulation in the MIS-C group, although these were also present in one minimal and one severe COVID-19 patient (Figure 3C & 3D). The trajectory of change in ALC and ANC was approximated using the slopes of linear regression models, with no overall significant difference between MIS-C and severe COVID-19 patients (Supplemental Figure S4B).

DISCUSSION

We report on a prospective cohort of pediatric patients representing the spectrum of disease associated with SARS-CoV-2 from minimal to severe COVID-19, and MIS-C, and identify biomarkers that distinguish different manifestations of the same infection. We have made several observations that improve understanding of disease biology and impact clinical care. The positive but high Cts associated with MIS-C support a post-infectious etiology for this phenomenon that has been previously postulated but not demonstrated.We describe clinical and laboratory measures that discriminate between patients with MIS-C and those with severe COVID-19, specifically the combination of the cytokine levels for IL-10 and TNF-α, and the presence of burr cells and toxic granulation on peripheral blood smears. While clinical cytokine

profiling is limited to tertiary care centers and central laboratories, peripheral blood smears are easily made in most clinics and hospitals.

Although initial reports have emphasized similarities between MIS-C and KD, consistent with our initial hypothesis, our findings suggest that MIS-C is a distinct entity that may have some overlapping features with KD. In our cohort, only two of six children with MIS-C met criteria for complete KD. As has been reported by other groups, most patients in our MIS-C cohort had cardiac dysfunction at presentation, a finding that is unusual in KD.(5, 6) Conversely, despite their dramatic presentations, there was very limited coronary artery disease in MIS-C patients. The cytokine profiles associated with the MIS-C group, particularly marked elevations in IL-10, are distinct from previously reported cytokine profiles in KD which tend to be associated with mild elevations of IL-1, IL-2, and IL-6.(19) Differences between our cohort and previously published cohorts must be interpreted with caution due to methodologic differences such as differences in platform, substrates used and timing of collection in disease course. However, TNF-α appears to play a key role in the pathogenesis of both MIS-C and KD.(19, 20) Levels of IFN-γ, a cytokine not strongly associated with KD, were very high in many patients with SARS-CoV-2 infections, including children with minimal disease. There was wide variability of IFN-γ levels in patients included in the cohort implying that IFN-γ signaling may be important in disease biology of COVID-19 and MIS-C, but is not necessary for development of either phenotype.

We also noted increases in markers of endothelial dysfunction in both MIS-C and severe COVID-19 with elevated sC5b-9 and altered red blood cell morphology. These findings correlated with elevations in the inflammatory cytokines IL-6, IL-8, and TNF-α, implying a connection to significant dysfunction of innate immunity. Further characterization of broader

panels of cytokines, particularly sIL-2R, IL-18 and CXCL9 would be valuable and of interest. Research focused on characterizing these cytokines and on defining causal relationships is necessary. Other clinical markers also differed between the two groups, including markers of vascular injury, such as elevated d-dimers and B-natriuretic proteins and markers of coagulopathy such as prolonged PTT. Per institutional protocols, some patients in both the COVID-19 and MIS-C group would have received prophylactic enoxaparin. Further studies will need to better characterize these differences between MIS-C and severe COVID-19.

Contrary to early reports that emphasized relatively mild disease outcomes in pediatric patients, we show that in some children SARS-CoV-2 appears to trigger a dysregulated hyperinflammatory pathophysiologic process.(2, 3) Strikingly, all children with MIS-C in our cohort were previously healthy in contrast to those in the severe COVID-19 cohort who had significant comorbidities, similar to disease in adult populations. A larger number of patients in our cohorts had ARDS based on what would be expected from previous reports of pediatric patients with respiratory COVID-19.(1-3, 21) In a report of pediatric patients with severe COVID-19 from Wuhan, only 2 patients required mechanical ventilation. The laboratory findings associated with severe COVID-19 presented in that report are less deranged than those in our cohort.(21) Congruent with adult reports, we note that black patients may be at higher risk of more severe disease, with these patients being over-represented amongst those who developed ARDS.(22) The extent to which these observed differences are mediated by social determinants of health and healthcare inequalities versus genetic or epigenetic factors warrants future investigation.

The small number of patients in our study precluded more detailed statistical analysis and modelling. We lacked the power to detect subtle differences between the groups in immunologic

and cytokine changes. We additionally acknowledge the issues of multiplicity of analyses, particularly the cytokine correlations, for which our small dataset precludes a complete multivariate analysis. Future work with a larger sample size and multiple cohorts for replications, will allow for analysis similar to the analyses we performed on cytokines with other clinical and laboratory variables. Due to the pandemic, we were only able to collect blood samples on hospitalized children, and therefore could not include non-admitted patients with mild SARS-CoV-2 without comorbidities. We used a cohort of patients with minimal symptoms of disease as our control, however, these patients were all hospitalized with different comorbidities. Of note, two of these patients had sickle cell disease and may have fundamentally different hematologic parameters at baseline than other patients.

We believe that these results can inform hypotheses for future studies when larger cohorts are available, and we would caution against broad generalization of these results until such work is complete. Future work will need to examine why SARS-CoV-2 induces such a damaging inflammatory response in some children and not others, with a focus on potential genetic etiologies as this has the potential to guide therapy. In our cohort of six patients, all children with MIS-C improved after treatment with IVIG, aspirin and steroids. Optimal treatment strategies will need to be determined in clinical trials.

Pediatric patients with SARS-CoV-2 are at risk of developing severe inflammatory dysregulation with critical illness. The detailed evaluations we present begin to dissect the pathophysiologic processes in these mysterious complications of SARS-CoV-2 and open the door for targeted therapeutics. Examination of peripheral blood smears for evidence of schistocytes, burr cells and neutrophil toxic granulations, while awaiting cytokine profiling may aid in distinguishing between patients with MIS-C and severe COVID-19 and guide immunomodulatory therapy.

METHODS

Study Design and Population

We prospectively screened and enrolled patients admitted to the Children's Hospital of Philadelphia (CHOP) between April 3rd and May 15th 2020. Patients were enrolled if they had evidence of past or current SARS-CoV-2 infection with either SARS-CoV-2 positive RT-PCR in blood, stool or mucosa, or presence of serum immunoglobulin G to SARS-CoV-2. In order to prevent bias, patients were enrolled without regard to clinical presentation, and were only categorized after enrolment into the study.

Patients were categorized as having MIS-C if they had fever, clinically severe illness with multisystem organ involvement (>2 of cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological), no alternative plausible diagnosis and positive SARS-CoV-2 infection by RT-PCR, serology, or COVID-19 exposure within the 4 weeks prior to the onset of symptoms, per the CDC case definition of MIS-C.(7) Patients were categorized as "severe COVID-19" if they did not meet criteria for MIS-C and presented with a primarily respiratory process requiring an increase in positive pressure support above their baseline. Patients were classified as "minimal COVID-19" if they required hospitalization but did not otherwise meet criteria for MIS-C or severe symptoms. Patients in the MIS-C cohort were evaluated for complete and incomplete KD. Co-infections were identified by chart review for microbiologically proven infections that were deemed clinically significant by a panel of infectious disease physicians.

Data Collection

Clinical data were abstracted from the medical record onto standardized case report forms using a REDCap database and double-checked by a physician reviewer. Data collected included demographics, comorbid conditions, sources of co-infection, vasoactive infusions, mechanical ventilation, anti-infectives, immunomodulatory agents, and laboratory indicators of organ dysfunction or inflammation. Hematoxylin-eosin peripheral blood smears were independently examined by two reviewers (hematologist and hematopathologist). Presence of burr cells and schistocytes were scored using ordinal scales (burr cells: 1+, present but <25% cells; 2+, 25%-50% cells; 3+, 51-75% cells; 4+, >75% of cells; schistocytes: absent, rare, or occasional).

Blood Collection and Assays

Blood was collected at the first planned clinical blood draw after consent was obtained. Patients presented to our hospital at different time points in their clinical illness, and some patients were transferred from outside hospitals. For patients who remained admitted to the hospital, blood draws were obtained on a weekly basis after the initial collection.

Proinflammatory cytokine profiling

Quantification of 10 pro-inflammatory cytokines was performed using V-Plex Pro-inflammatory Panel 1 Human Kits (Cat. #K15049D; Meso Scale Diagnostics, Rockville MD, USA). Cytokines assayed were IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α. Samples were analyzed in duplicates and assays were performed per manufacturer protocol and read and analyzed on a QuickPlex SQ120 (Meso Scale Diagnostics).

Soluble C5b-9 assay

Triplicates of plasma samples were assayed for soluble C5b-9 (sC5b-9) levels at two dilutions by using human C5b-9 ELISA set (Cat. #558315; BD Biosciences, San Jose CA, USA) per manufacturer protocols. Standard curves were analyzed for best fit curves, and resulting equations were used to derive sC5b-9 sample concentrations.

Viral Cycle Thresholds

Viral cycle thresholds (Cts) for SARS-CoV-2 were measured in a Clinical Laboratory Improvement Amendments of 1988 - College of American Pathologist (CLIA-CAP) certified laboratory at CHOP using an RT-PCR assay for N2 viral RNA under Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA).

Statistics

Clinical characteristics were summarized using descriptive statistics. The analysis cohort was comprised of individuals for whom complete cytokine data were available. Analysis and visualizations were performed using Prism 8 (GraphPad, San Diego CA, USA) and Stata/IC (StataCorp, College Station TX, USA). Heatmaps were generated using Morpheus (https://software.broadinstitute.org/morpheus).

For comparisons between groups, non-parametric tests were used with post-hoc multiple comparison corrections when more than 2 groups were present. For the analyses presented in Supplemental Table S1, the Mann Whitney test was used to estimate approximate p values. Multiple corrections were not performed as the small sample size precluded this test. Two-way ANOVA was used to check for interaction effects of phenotype with size of unit vector

components for cytokines. For count data, Chi-squared testing was used, utilizing test for trends when ordinal variables were present. Univariate logistical regression was performed to determine correlations between continuous variables. A p-value less than 0.05 was considered significant.

Study Approval

The protocol was approved by the CHOP Institutional Review Board. Due to the COVID-19 pandemic, verbal informed consent was obtained from a legally authorized representative as per the Declaration of Helsinki. Written informed consent was signed by the consenting physician and a copy was provided to participants.

AUTHOR CONTRIBUTIONS

EMB, DTT, and HB equally contributed to this work and are co-senior authors. CD, SEH, LV, KOM, JC, EB, HB, and DTT designed the study, analyzed data, and wrote the manuscript; KES, CJ, KC, ML, MP, FB, DMB, BLB, KMB, AMB., BTF, JCF., JSG., KG, CG., SAG., RMH., TJK., ARJ., EJL, WP, CP, AFR., NDR., AS, DAS., and JV, contributed to study design, analyzed data, and helped write the manuscript, CB and JH performed experiments, provided data, and edited manuscript. CD, JC, and KOM enrolled subjects. All authors contributed intellectually and reviewed and revised the manuscript.

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Table 1. Demographic and clinical characteristics of enrolled patients.

	MIS-C	Non-MIS-C	
		Severe COVID-19	Minimal COVID-19
	N = 6	N = 9	N = 5
Age			
Median (IQR)	6 (5,7)	16 (14,17)	13 (4,16)
Gender N (%)			
Female	4 (67)	5 (56)	2 (40)
Male	2 (33)	4 (44)	3 (60)
Race N (%)			
White	4 (67)	2 (22)	3 (60)
Black	1 (17)	5 (55)	2 (40)
Other	1 (17)	1 (11)	0
Declined	0	1 (11)	0
Ethnicity N (%)			
Hispanic	1 (17)	1 (11)	0i
Not Hispanic	5 (83)	7 (78)	5 (100)
Declined	0	1 (11)	0
PICU Admission			
Yes	5 (83)	9 (100)	1 (20) *
No	1 (17)	0	4 (80)
ECMO			
Yes	0	2 (22)	0
No	6 (100)	7 (78)	5 (100)
Inotropic Support			
Yes	5 (83)	7 (78)	0
No	1 (17)	2 (22)	5 (100)
Max Respiratory Support			
None	1 (17)	0	5 (100)
Low flow	1 (17)	0	0
CPAP	1 (17)	0	0
BiPAP	1 (17)	1 (11)	0
Intubation	2 (33)	8 (89)	0
ARDS			
Yes	1 (17)	5 (56)	0
No	5 (83)	4 (44)	5 (100)
AKI**			
Yes	3 (50)	4 (44)	0
No	3 (50)	5 (56)	5 (100)
Coinfections			
Yes	1 (17)	4 (44)	2 (40)
No	5 (83)	5 (56)	3 (60)
Lymphopaenia			
Yes	6 (100)	8 (89)	2 (40)
No	0	1 (11)	3 (60)
Neutropenia			
Yes	0 (0)	3 (33)	1 (20)
No	6 (100)	6 (67)	4 (80)

*one patient was admitted prophylactically to the PICU for increased monitoring of seizures **defined as creatinine >1.5x ULN; ARDS – acute respiratory distress syndrome; AKI – acute kidney injury; ECMO – extracorporeal membrane oxygenation; PICU – pediatric intensive care unit.

Table 2. Clinical presentations of enrolled patients.

ID	Age (yrs.)	Sex	Comorbidities	Brief summary of Presentation	Co-Infection	Outcome
Patients with MIS-C						
12	5	M	Previously healthy	Fever, dehydration, severe abdominal pain, rash, conjunctivitis, and swollen hands.		Recovered
18	6	F	Previously healthy	Fever, rash, cracked lips, conjunctivitis, myocardial dysfunction and shock, requiring intubation and vasoactives.		Recovered
19	5	F	Previously healthy	Fever, shock, conjunctivitis, and cracked lips, myocardial dysfunction requiring vasoactives.	Parainfluenza III, IV	Recovered
22	6	F	Previously healthy	Fever, emesis, severe abdominal pain, myocardial dysfunction and shock, requiring intubation and vasoactives. Developed ARDS.		Recovered
24	7	F	Previously healthy	Fever, conjunctivitis, cracked lips, abdominal pain, diarrhea, myocardial dysfunction, and shock, requiring BIPAP and vasoactives.		Remains on inotropes
26	13	M	Previously healthy	Fever, diarrhea, abdominal pain, shortness of breath, dark urine, myocardial dysfunction, and shock, requiring CPAP and vasoactives.		Recovered
Patients with severe COVID-19						
1	17	M	R/R B-ALL	Fever, septic shock, ARDS, requiring intubation and vasoactives.	*E. coli* BSI, osteomyelitis	Death
4	18	F	Hypertension, IDDM, HCM	Fever and ARDS, requiring ECMO.		Death
7	0.17	M	Prematurity (29 weeks)	Apnea, cyanosis, hypothermia, and hypotension, requiring intubation.	Enterovirus	Recovered
10	10	M	Craniopharyngioma in remission, hypopituitarism, branchial cleft cyst	Acute respiratory failure and shock, requiring intubation and vasoactives.	Osteomyelitis, adenovirus, rhinovirus	Recovered
15	15	F	Epilepsy, dystonia, asthma, thoracic insufficiency, OSA	Fever, acute respiratory failure and shock, required vasoactives. Developed ARDS.		Remains intubated
17	17	F	HIE, hypopituitarism, epilepsy, prev. tracheostomy	Acute respiratory failure, and shock, requiring intubation and vasoactives. Developed ARDS.		Remains intubated
20	16	F	CP, recurrent UTI, contractures, tracheostomy	Tactile fever and increased oxygen demand, requiring intubation.	*E. coli* UTI	Recovered
23	18	M	CP, home BiPAP, adrenal insufficiency, hypoglycemia	Cough, lethargy, and hypotension, requiring escalation of BIPAP.		Recovered
25	14	F	Prematurity (27 weeks); no sequelae of prematurity	Fever and ARDS, requiring ECMO.		Remains intubated
Patients with minimal COVID-19						
6	2.5	M	Previously healthy	Shoulder pain and extra-articular abscess.	MRSA BSI, osteomyelitis	Recovered
11	15	F	Epilepsy, CP, global developmental delay	Prolonged tactile fever and increased seizure frequency.		Recovered
13	4	M	SCD-SS	Fever and diarrhea.	*S. typhi* enteritis	Recovered
14	18	M	Osteosarcoma	Ground glass opacities on routine screening.		Recovered
16	13	F	SCD-SS	Cough, admitted for prophylactic transfusion with sickle cell and COVID-19.		Recovered

ARDS – acute respiratory distress syndrome; B-ALL – B-cell acute lymphoblastic leukemia; BSI – blood stream infection; CP – cerebral palsy; ECMO – extracorporeal membrane oxygenation; *E. coli – Escherichia coli*; GH – growth hormone; HCM – hypertrophic cardiomyopathy; HIE - hypoxic ischemic encephalopathy; IDDM – insulin dependent diabetes mellitus; MRSA – methicillin-resistant *Staphylococcus aureus*; R/R – relapse refractory; *S. typhi – Salmonella typhi;* SCD-SS – Sickle cell disease, hemoglobin SS; UTI – urinary tract infection;

Table Legends

Table 1. Demographic and clinical characteristics of enrolled patients.

Table 2. Clinical presentations of enrolled patients.

Figure 1. Cytokine architecture associated with SARS-CoV-2 infections in children. A) Heat map of the 5 most differentially present cytokines in the plasma of pediatric SARS-CoV-2 infections. Comparison of patients with and without co-infections for each of the three clinical phenotypes of pediatric SARS-CoV-2 infection (N=20). Patient IDs are listed above each column for reference. B) Cytokine profiles for each patient (N=15) were treated as a 5-dimensional vector and converted into unit vectors by dividing each component by the root sum square of the vector. Box and whisker plot of each unit vector with median values of each for MIS-C versus severe COVID-19 presentations (line). Whiskers represent maximum and minimum and boxes the 25th to 75th percentile. Differences between phenotypes or phenotype/cytokine interaction were not significant by two-way ANOVA. C) Vector magnitudes of each cytokine profile were computed as the root sum square with values plotted for severe COVID-19 and MIS-C phenotypes (N=15). Bar represents the mean of each group. Differences were not significant by Mann-Whitney U test. D) The sum of the IL-10 and TNF-α concentrations were computed for each patient and plotted for severe COVID-19 and MIS-C phenotypes (N=15). Bar represents the mean of each group with p-values computed by Mann-Whitney U test.

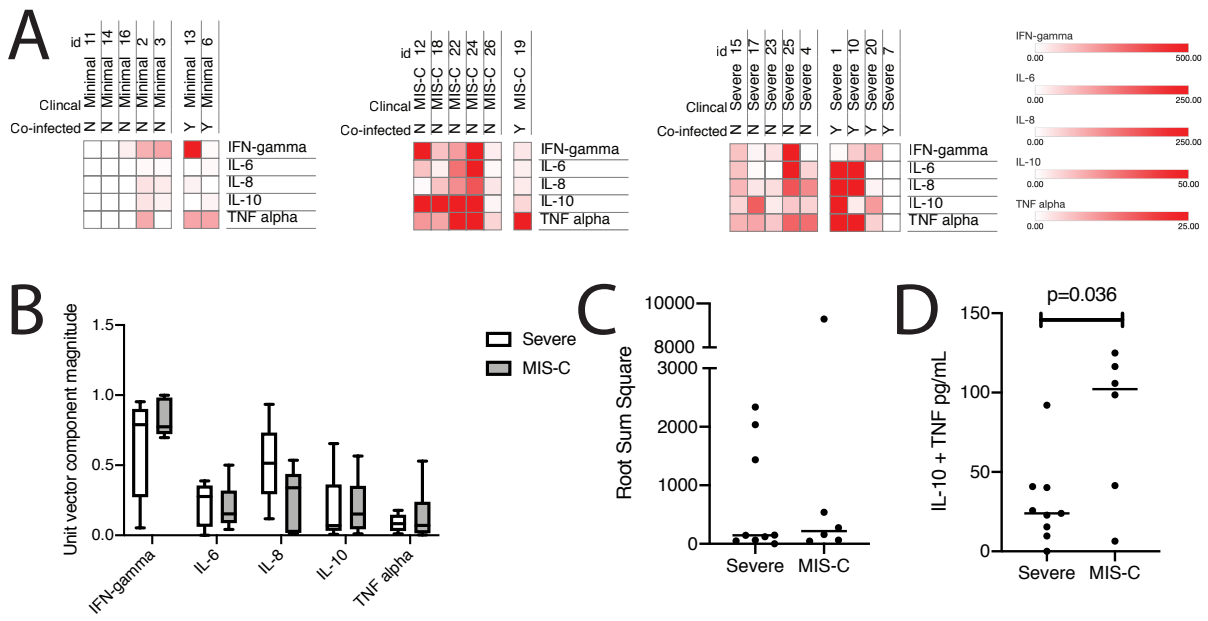


Figure 2. Viral cycle thresholds (Cts) in pediatric SARS-CoV-2 infections. A) When available (N = 16), Cts for each patient are plotted. Bars represent mean values with p-values computed using Dunn's multiple comparisons test after Kruskal-Wallis testing. B) Linear regression modelling of cycle time thresholds against IL-10 and TNF-α concentrations in plasma for all patients in the cohort (N=16). n.s. represent non-significant correlation.

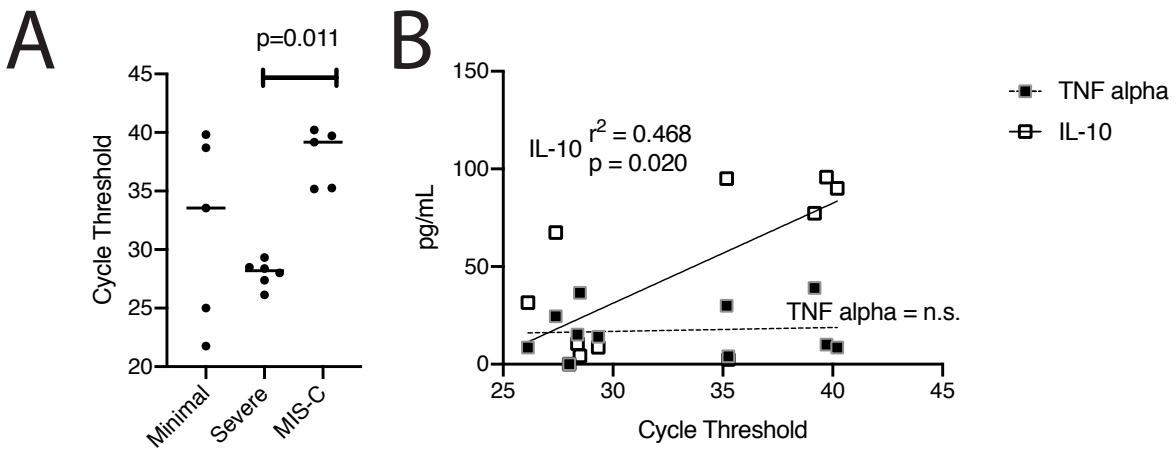
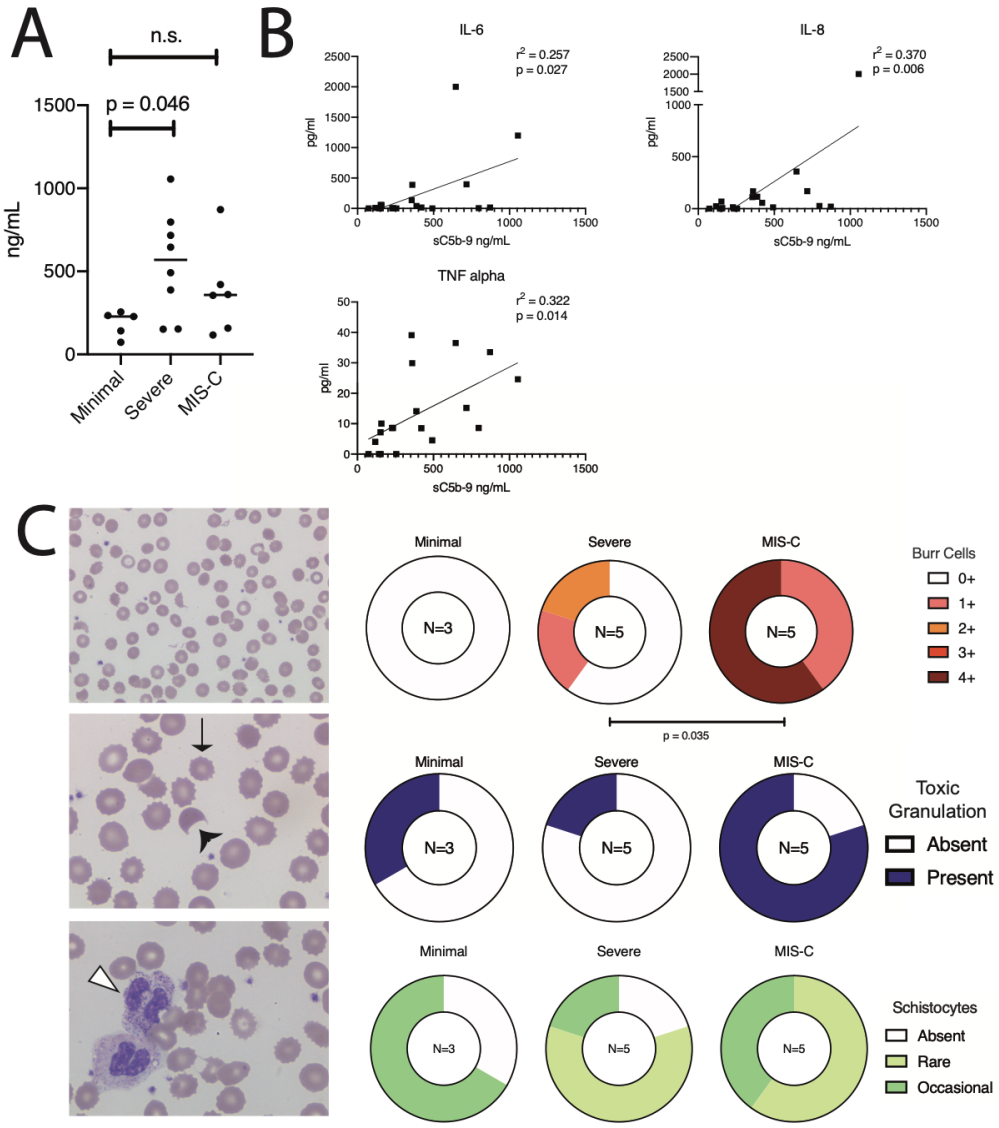


Figure 3. Soluble C5b-9 measurements in pediatric SARS-CoV-2 infections. A) Soluble C5b-9 was successfully measured in N=19 patients. Data are plotted for each of the 3 phenotypes, bars represent the mean values of each group. p-value computed using Dunn's multiple comparisons test after Kruskal-Wallis testing, n.s. represents non-significant value. B) Linear regression modelling of soluble C5b-9 against IL-6, IL-8, and TNF-α concentrations in plasma for all patients in the cohort. C) Representative photomicrographs of red blood cells and neutrophils from patients in the cohort. From top to bottom: 50X field showing burr cells and schistocytes; 100X field showing the same; 100X field showing toxic granulation. Thin arrow denotes a burr cell, black arrowhead denotes a schistocyte, white arrowhead denotes toxic granulation. D) Number of patients with degree of burr cells, toxic granulation, and schistocytes for each clinical phenotype; N=13 patients with smears available. Severe COVID-19 was compared to MIS-C using Chi-squared test for trend for burr cells, Chi-squared statistic was impossible to compute for the dichotomous outcome of toxic granulation due to low N. There were no statistically significant differences in schistocytes across the cohort.



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