Distinct Cytokine and Chemokine Dysregulation in Hospitalized Children with Acute COVID-19 and Multisystem Inflammatory Syndrome with Similar Levels of Nasopharyngeal SARS-CoV-2 Shedding.

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Summary: A prospective cohort study, where similar levels of nasopharyngeal viral RNA were found in children with MIS-C and acute COVID-19, despite finding significantly elevated cytokine/chemokine levels in MIS-C patients, after adjusting for age and sex.

ABSTRACT

Background: Multisystem Inflammatory Syndrome in Children (MIS-C) is a severe clinical phenotype of SARS-CoV-2 infection that remains poorly understood.

Methods: Hospitalized children <18 years of age with suspected COVID-19 (N=53) were recruited into a prospective cohort study; 32 had confirmed COVID-19, with 16 meeting the U.S. Centers for Disease Control criteria for MIS-C. Differences in nasopharyngeal viral RNA levels, SARS-CoV-2 seropositivity, and cytokine/chemokine profiles were examined, including after adjustments for age and sex.

Results: The median ages for those with and without MIS-C were 8.7 years (IQR 5.5-13.9) and 2.2 years (IQR 1.1-10.5), respectively, (p=0.18) and nasopharyngeal levels of SARS-CoV-2 RNA did not differ significantly between the two groups (median 63,848.25 copies/mL versus 307.1 copies/mL, p= 0.66); 75% of those with MIS-C were antibody positive compared to 44% without, p=0.026. Levels of 14 of 37 cytokines/chemokines (IL-1RA, IL-2RA, IL-6, IL-8, TNF-α, IL-10, IL-15, IL-18, MCP-1, IP-10, MIP-1α, MCP-2, MIP-1β, Eotaxin) were significantly higher in children with MIS-C compared to those without, irrespective of age or sex (FDR<0.05; p<0.05).

Conclusions: The distinct pattern of heightened cytokine/chemokine dysregulation observed with MIS-C, compared with acute COVID-19, occurs across the pediatric age spectrum and with similar levels of nasopharyngeal SARS-CoV-2 RNA.

Key Words: SARS-CoV-2; MIS-C; MIS-A; Coronavirus; COVID-19; viral RNA

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the novel coronavirus first identified in Wuhan, China in 2019, maintains high levels of circulation with over 155,000,000 cases worldwide as of May 6, 2021[1-3]. Initial reports from China of SARS-CoV-2 infection in children described a mild disease consisting mainly of fever, upper respiratory tract illness, and gastrointestinal symptoms[4, 5]. Later, case series from Europe described a syndrome of systemic inflammation akin to Kawasaki Disease (KD)[6] with features of toxic shock, referred to as Paediatric Multisystem Inflammatory Syndrome[7]. Similar cases were subsequently identified in the US and named Multisystem Inflammatory Syndrome in Children (MIS-C)[8-10]. A similar inflammatory syndrome associated with SARS-CoV-2 was recognized in adults and referred to as Multisystem Inflammatory Syndrome in Adults (MIS-A)[11].

MIS-C is reported as largely post-infectious and mostly in older children (median age 8 years)[12] with the acute phase showing elevations in proinflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, and IFN-γ)[13, 14]. Although several studies have reported elevations in cytokines with MIS-C, overlapping those seen in severely affected adult patients[15], few pediatric studies have adjusted for age and sex, or have examined quantitative differences in nasopharyngeal virus shedding in acute COVID-19 compared with MIS-C to establish the interaction between the patterns of cytokine elevations and disease phenotype.

Further knowledge regarding the patterns of cytokine elevation and disease phenotype while adjusting for factors such as age and sex, as well as the viral RNA levels of children with acute COVID-19 and MIS-C will help to further understand the immunopathogenesis of SARS-CoV-2 infection and MIS-C. From a prospective cohort study established at an urban

Children's Center at the outset of the U.S. pandemic, we report the quantitative viral RNA and cytokine/chemokine levels of children hospitalized with COVID-19 across the age-spectrum, comparing those with acute COVID-19 and MIS-C.

METHODS

Study participants. Children admitted with suspected SARS-CoV-2 infection to The Johns Hopkins Hospital, an academic medical center in Baltimore, Maryland, were prospectively enrolled into a cohort study of children and adults with suspected COVID-19, "Clinical Characterization Protocol for Severe Emerging Infections" (CCPSEI). Data from the study participants enrolled from April 10, 2020, to July 10, 2020, are summarized.

Informed consent and sample acquisition. The study was approved by the Johns Hopkins Medicine Institutional Review Board. After informed consent, blood samples were collected in coordination with routine medical care, and/or remnant blood and nasopharyngeal samples were retrieved from the clinical laboratory. Participants transferred from outside hospitals did not have remnant samples available.

Clinical data. Review of medical records was performed by manual chart extraction for demographic and clinical data. Participants were considered to have confirmed SARS-CoV-2 infection if they had either: positive nucleic acid test (NAT); negative NAT with positive serology; or negative NAT and serology, but history of exposure to a confirmed COVID-19 contact within one month of admission. Participants were defined as having MIS-C using the CDC case definition: individuals <21 years with fever, laboratory evidence of inflammation, severe illness requiring hospitalization, involvement of greater than two organ systems, no

alternative plausible diagnoses, and positive for current/recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected/confirmed COVID-19 case within 4 weeks of symptom onset[10].

Virologic Diagnosis and Viral RNA Quantitation. Infection with SARS-CoV-2 was based on positive NAT by any of the 7 clinically validated real-time polymerase chain reaction (RT-PCR) assays available at The Johns Hopkins Hospital. SARS-CoV-2 viral RNA levels were quantified using digital droplet PCR (Bio-Rad, Hercules, CA) on RNA extracted from remnant viral transport media (VTM). Primers targeting the nucleocapsid regions N1 and N2 were used. The estimated limit of quantitation was 1.25 copies per milliliter (mL) of VTM.

Serological Testing. Serologic testing was performed in participants either as part of clinical care or on archived serum using the Euroimmun enzyme-linked immunosorbent assay (Mountain Lakes, NJ) detecting IgG targeting the S1 domain of the SARS-CoV-2 spike protein with a limit of detection of 1.23 units. Seropositivity is reported as a unitless ratio by dividing the optical density by the assay calibrator[16].

Cytokine/Chemokine Profiling. Serum cytokines/chemokines were measured using the Meso Scale Discovery V-plex 30 kit which allows quantification of 21 cytokines (GM-CSF, IFN-γ, IL-10, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-23p40, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8(HA), TNF-α, TNF-β, VEGF) and 9 chemokines (Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC), to which 7 additional cytokines (IL-18, IL-1RA, IFN-λ, IL-23, IL-2RA, IFN-α2, TNF-β) and 1 chemokine (MCP-2) were added. Assays were performed according to the manufacturer's protocol, and data were acquired on a MESO QuickPlex SQ 120. Testing was only performed on participants with freshly

collected blood on Day 0 of study enrollment (N=19). In one participant who succumbed to SARS-CoV-2 infection, a remnant sample was used.

Statistical analyses. Comparison of the distribution of categorical variables between those with acute COVID-19 and MIS-C was performed using Fisher's exact test, and comparisons of continuous variables were evaluated using non-parametric rank sum test. Logistic regression analyses were used for odds ratios and confidence intervals. Data were analyzed using R; significance was determined by $p<0.05$. To evaluate differences in length of stay, viral load (log-transformed), IgG titer, and cytokines/chemokines (log-transformed), between children with acute COVID-19 and MIS-C, after adjusting for age and sex, a linear regression was fitted for each of these dependent variables using disease status, age, and sex as independent variables. P-values for disease status were obtained to indicate whether each dependent variable was statistically different between acute COVID-19 and MIS-C status. For cytokines, p-values for disease status were converted to Benjamini-Hochberg false discovery rates (FDR)[17]. A significant association between cytokines and disease status was determined by FDR<0.05. We have also applied similar analyses to compare the cytokine/chemokines between MIS-C and unconfirmed, and between COVID-19 and unconfirmed. Similarly, cytokines associated with age or sex were identified by linear regression using disease status, age, or sex as independent variables; a relaxed FDR of <0.25 was used to determine significance for age or sex, due to the sample size. To test whether the association between cytokine and disease status was different for different age or sex, linear regression was fitted using disease status, age, sex, and interaction between disease status and age or sex as independent variables. A significant interaction was determined by a relaxed FDR of <0.25, due to sample size. Figures were generated with GraphPad Prism 8.4.3 and R.

RESULTS

Demographics of the Overall Cohort. Of the 53 participants, 32 met study criteria with confirmed SARS-CoV-2 infection (Supplemental Figure 1). The median age was 7.4 years (IQR 1.6-13.9 years, range: 13 days-20 years); 25% were black and 50% were Hispanic (Table 1). The cohort was evenly divided between males and females. For the 21 children without confirmed SARS-CoV-2 infection, the median age was 3.45 years (IQR 1.4-6.19 years) which was not significantly different from those with confirmed infection (p=0.241); their clinical and laboratory data are summarized in Supplemental Table 1.

Demographic, Clinical, and Laboratory Features by Disease Status. Fifty percent (N=16) met CDC criteria for MIS-C (Supplemental Figure 1). The median ages were 8.72 years (IQR, 5.45-13.93 years, range: 3 months-17.5 years) and 2.24 years (IQR 1.09-10.5 years, range: 13 days-20 years) for children with MIS-C and acute COVID-19, respectively (p=0.175). A higher proportion (63%) of children with MIS-C were Hispanic compared to those without (37.5%). Fifty-six percent of children with MIS-C were male. Body-mass index percentile was not significantly different between children with and without MIS-C. Children with MIS-C were significantly more likely to present with gastrointestinal (88% versus 44%, p=0.023), mucocutaneous (63% versus 13%, p=0.009), and musculoskeletal symptoms (31% versus 0%, p=0.043) compared to those acute COVID-19. The median duration of symptoms prior to presentation was similar in both groups (3 versus 4 days, p=0.494) as was the median length of stay (6 versus 5 days, p=0.835) (Table 1 and Supplemental Table 2). This finding was the same even after adjusting for age and sex, although the median length of stay for those with MIS-C and acute COVID-19 was

significantly longer than the median length of stay for those with unconfirmed SARS-CoV-2 infection (3 days, p=0.004 and p=0.003, respectively) (Supplemental Figure 2).

Nucleic acid testing (NAT) was positive in 50% of children with MIS-C and 75% of those with acute COVID-19 (p=0.27). The median viral load in the children with MIS-C was 63,848.25 copies/mL (IQR 461.38->1,254,000; range <1.25->1,254,000) and 307.1 copies/mL (IQR <1.25->1,254,000; range <1.25->1,254,000) in those with acute COVID-19 (p= 0.66) (Figure 1A). More MIS-C patients were seropositive for SARS-CoV-2 when compared to those with acute COVID-19, (75% versus 44%, p=0.026) (Figure 1B).

Overall, MIS-C patients had higher acuity than those with acute COVID-19 (Table 1). Fifty-six percent (N=9 of 13) of MIS-C patients had abnormal echocardiograms showing: decreased left ventricular function/ventricular strain (N=5), coronary artery dilatation (N=4); one child with coronary artery dilatation also had a pericardial effusion. The two children without MIS-C had normal echocardiograms. One participant, a female adolescent, succumbed to MIS-C after 5 days of hospitalization from cardiogenic shock. This participant who was previously reported was 15 years old and presented early in the pandemic in May 2020, with a one-week history of worsening epigastric pain without nausea or diarrhea. She progressively developed worsening hypovolemic shock and left ventricular dysfunction requiring vasopressors; An autopsy revealed diffuse lymphoplasmacytic inflammatory infiltrate in the septum of the heart and inflammation of the small arterioles of the heart. There was no evidence of inflammation in the lungs. Two NATs for SARS-CoV-2 were negative at presentation, but a third NAT on hospital day 5 was positive, and replication competent virus was recovered in a research laboratory. Her SARS-CoV-2 serology was reported on hospital day 5 as positive.

Laboratory Findings. Laboratory tests on admission are summarized in Supplemental Table 3. As in other studies, the children with MIS-C had significantly lower absolute lymphocyte counts (ALC; median 770; IQR 395-1645), platelet counts (median 171K, IQR 85-253K) , and significantly higher C-reactive protein (CRP) levels on admission than those without (12.2 mg/dL versus 0.95 mg/dL, p=0.001). Participants who presented with an ALC <1500 or platelet count <250K were more likely to have MIS-C (OR 9.53 [95% CI 2.54-41.56] and OR 3.8 [95% CI 1.08-14.88], respectively). In our study, cardiac biomarkers, such as troponin and pro-Brain natriuretic protein, did not differ significantly between the two groups.

Cytokine/Chemokine Profiles and Associations, after adjusting for Age and Sex. Of the 37 cytokines and chemokines measured, 10 cytokines (IL-10, IL-15 , IL-16, IL-18, IL-1RA, IL-2RA, IL-6, IL-8, TNF-α, VEGF) and 7 chemokines (Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-2, MIP-1α, MIP-1β) were significantly higher ($p<0.05$) in children with MIS-C compared to those with acute COVID-19 (Figure 2A, Supplemental Table 4). After adjusting for age and sex, 8 of the cytokines (IL-1RA, IL-2RA, IL-6, IL-8, TNF-α, IL-10, IL-15, IL-18) and 6 of the chemokines (MCP-1, IP-10, MIP-1α, MCP-2, MIP-1β, Eotaxin) were significantly elevated in MIS-C patients (FDR<0.05, $p<0.05$). No significant differences in cytokine levels were observed with age and disease status (FDR>0.25, $p>0.05$)(Figure 2B)(Supplemental Table 5). Regardless of disease status, MCP-2 was found to be positively correlated with age (FDR<0.25, $p<0.05$). Independent of disease status, females with SARS-CoV-2 infection had significantly higher levels of IL-6 and TNF-α compared with males (FDR<0.25, $p<0.05$). Levels of IL-6, IL-10 and IL-2RA also tended to be higher in females compared to males with MIS-C (FDR>0.25, $p<0.05$) (Figure 2C, Supplemental Table 5),

however, no significant differences in cytokines were observed when sex and disease status were combined (FDR>0.25, p>0.05). Comparisons between the participants with confirmed SARS-CoV-2 infection to those unconfirmed are summarized in Supplemental Figure 3 and Supplemental Table 6.

DISCUSSION

In this prospective cohort study established early in the U.S. pandemic among children hospitalized with COVID-19 at a single urban center, we evaluated presence of viral RNA as well as cytokine/chemokine profiles in pediatric patients infected with SARS-CoV-2, comparing acute COVID-19 to MIS-C, including those with clinical signs and symptoms of acute COVID-19 but with unconfirmed SARS-CoV-2 infection. Our cohort of children with MIS-C had significantly higher odds of lymphopenia (10-fold) and thrombocytopenia (4-fold), and higher levels of CRP, confirming appropriate disease stratification of the participants during the early phases of the pandemic in the U.S. We found similar, levels of SARS-CoV-2 RNA in the nasopharynx at presentation amongst those with acute COVID-19 and those with MIS-C despite significant differences in the cytokine/chemokine levels of these patients, and seropositivity status, after adjusting for age and sex. Some MIS-C participants had levels of nasopharyngeal viral RNA above the upper limit of the assay (>1,254,000 copies/mL) at presentation.

Given the age and sex-related differences in immune responses to viral infections in children, cytokine and chemokine differences were also examined after adjusting for disease status. Except for MCP-2 which showed a positive correlation with increasing age, no age-related differences were observed in this small cohort. Female sex was found to be associated with higher levels of IL-6 and TNF-α with SARS-CoV-2 infection. However, explorations of the

associations between cytokine/chemokine elevations and the combined factors of either age and disease status or sex and disease status showed no significant association, and only trends in elevations of IL-6, IL-10 and IL-2RA were seen as a function of female sex and disease status, which would require study in larger cohorts.

In general, MIS-C is considered a post-infectious process[12, 18-21] and indeed 75% of the children with MIS-C in our cohort were seropositive on admission compared with 44% of those with acute COVID-19. Interestingly, 50% of the children with MIS-C in our cohort had a positive NAT with viral loads similar to those with acute COVID-19, and the median copy number was higher in the MIS-C group. The current CDC definition of MIS-C includes SARS-COV-2 positivity by RT-PCR as criteria for diagnosis. Additionally, previous studies and larger cohorts have evaluated the detection of viral RNA by NAT in patients with MIS-C and have noted as many as half or more of patients admitted with suspected or confirmed MIS-C in their cohorts, to have tested positive[8, 22]. Few studies, however, have looked at the viral load in these patients to quantify the viral RNA detected to aid with distinguishing low-level virus shedding from possible replicating virus. In one study of 18 patients with MIS-C, 2 (11%) had quantifiable viral RNA in the nasopharynx[23], indicating that a proportion of children with MIS-C can be identified with nucleic acid testing and can still have quantifiable levels of SARS-CoV-2 viral RNA present at the time of presentation. Although viral culture was not routinely performed in our study or the Yonker et al. study to assess infectiousness, studies have found correlations between cycle-thresholds in real-time PCR based NATs with recovery of replication competent virus[24, 25]. Interestingly, one patient in our cohort who succumbed to MIS-C, did have replication competent virus recovered[26]. Inclusion of viral load quantitation and its correlation with replicating virus may inform the immunopathogenesis and management of MIS-C, as well as expand the

possibility for consideration of antivirals and neutralizing antibodies in the acute management of MIS-C.

Regardless of age or sex, after evaluating an extensive panel of cytokines and chemokines, we observed 14 inflammatory cytokines (IL-1RA, IL-2RA, IL-6, IL-8, TNF-α, IL-10, IL-15, IL-18) and chemokines (MCP-1, IP-10, MIP-1α, MCP-2, MIP-1β, Eotaxin) that were significantly elevated in correlation with MIS-C compared with acute COVID-19, despite similar duration of symptoms, length of hospital stay and nasopharyngeal viral RNA levels, highlighting the unique host inflammatory responses responsible for MIS-C. Our findings of cytokine/chemokine dysregulation with MIS-C are consistent with previous reports[18, 22, 27-29]. In one study, 16 children with MIS-C, compared to acute COVID-19, had significantly higher levels of seven cytokines (IL-8, IL-6, IFN-γ, IL-17A, IL-6, TNF-α, IP-10)[30]; and elevated levels of six cytokines (IL-1β, IL-6, IL-8, IL-10, IL-17, IFN-γ) was observed in a cohort of 25 children with MIS-C[13]. Likewise, Cheung et al. also reported in 8 children with MIS-C, elevated levels of IL-2R, IL-18, and CXCL 9 levels and mildly increased IFN and IL-8 in less than half, but normal levels of TNF-α[31]. Compared with these studies, ours included a broader panel of 37 cytokines/chemokines to compare between the two disease phenotypes (hospitalized children with acute COVID-19 and MIS-C) that was also used to study the pathobiology of severe SARS-CoV-2 infection in adults at the same urban care center[15]. In the adult study, IL-18, IL-6, and TNF-α were significantly elevated in adults with severe COVID-19, highlighting similarities in innate immune response pathways in severe COVID-19 in both children and adults[15].

The 14 cytokines/chemokines we identified that distinguished between MIS-C and acute COVID-19 reflect innate immune system activation, particularly of the monocyte/macrophage lineage[32], which are important for generating the adaptive immune responses, especially against viral pathogens[33]. Interestingly, significant elevation of IL-6,

TNF-α, IP-10, MCP-1, MCP-2, MIP-1α, and MIP-1β seen in MIS-C patients is also reported in macrophage activation syndrome (MAS)[34]. Together with the high SARS-CoV-2 RNA detected in the nasopharynx of patients with MIS-C, these findings warrant further exploration. Improved understanding of the host-specific cytokine/chemokine dysregulation implicated in the immunopathogenesis of SARS-CoV-2 infection may also aid in identifying targeted immunotherapeutic approaches as it has with KD[35]. For example, targeted anti-cytokine therapy has been utilized in the management of KD, with previous studies exploring anti-TNF-α for those high risk for coronary artery aneurysm development, as well as current AHA guidelines recommending infliximab or anakinra for IVIG-resistant disease[6].

In regards, to age and sex correlations, our small sample size revealed primarily exploratory findings. The proinflammatory chemokine, MCP-2 was found to be positively correlated with age, irrespective of MIS-C status. As this cytokine was also separately noted to be associated with disease status, irrespective of age or sex, it could raise the possibility of MCP-2 as an important biomarker of MIS-C. MCP-2 stimulates and attracts granulocytes and mononuclear cells, and previously was studied as a biomarker for tuberculous disease where a simple ELISA-based assay was used to accompany a clinically utilized IFN-γ assay (QuantiFERON TB® Gold In-Tube) in efforts to increase sensitivity of detecting clinically severe patients[36]. Regarding sex, male adults with COVID-19, generally have more severe outcomes[37], although one study demonstrated that higher levels of innate immune-mediated cytokines (IL-8 and IL-18) in women can be associated with more severe disease progression[38]. We noted higher levels of IL-6 and TNF-α in females compared with males with SARS-CoV-2 infection, independent of disease status, and higher levels of IL-6, IL-10 and IL-2RA in females with MIS-C compared with males, though these findings are limited by the small sample size. Sex differences in cytokine responses to SARS-CoV-2 infection in

children have not been reported and warrant further investigation in larger cohorts of children.

There are several limitations to this study. First, this was a single-center, observational cohort, and our data could be influenced by selection bias that reflects the demographic of our patient population, which is predominantly black and Hispanic. It should be noted, however, that Baltimore's population primarily consists of individuals who identify as black (63%) and those identifying as Hispanic account for only ~6% of the population[39]. It remains unclear why our cohort was so disproportionately Hispanic, but national reports share similar findings of higher prevalence of MIS-C in Hispanic children[40], and further studies investigating the disparities in this disease are necessary[41]. Additional limitations include the small sample size due to the relatively mild disease course of SARS-CoV-2 infection in children, rarity of MIS-C, and stringent SARS-CoV-2 diagnostic criteria[42] to eliminate confounders, thus excluding 21 suspected but unconfirmed cases in our cohort. In these 21 children presenting with an unspecified viral infection, cytokine dysregulation was also not observed, highlighting the unique host response to SARS-CoV-2 in the subset of children who develop MIS-C associated with high risk for cardiovascular compromise and currently unknown long-term sequelae.

In conclusion, our prospective cohort study in which participants were sequentially enrolled in real-time during the pandemic, further characterizes the unique cytokine/chemokine profiles of children with MIS-C compared to acute COVID-19, irrespective of age or sex, along with information on nasopharyngeal viral RNA levels in MIS-C. Additionally, potential sex differences in cytokines with SARS-CoV-2 infection, were observed, and these altogether add to the understanding of this severe clinical phenotype arising from SARS-CoV-2 infection in children[8, 18, 22, 26, 28] with possible implications on approaches to diagnosis and management.

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Tables and Figures Legend:

Table 1. Demographic, clinical, virologic and immunologic characteristics of children with acute COVID-19 or with MIS-C.

Figure 1. SARS-CoV-2 nasopharyngeal viral RNA copy number and antibody status in children with acute COVID-19, with MIS-C, and those without confirmed SARS-CoV-2 infection. Statistically significant values are denoted with a star (*). The dark line indicates the median. (A) Nasopharyngeal SARS-CoV-2 viral RNA (log_2(copies/mL)) to N1 of SARS CoV-2 in children with MIS-C (N=6), with acute COVID-19 (N=13), and unconfirmed (N=11). Similar values were seen for N2 (data not shown). Values at zero denote those that are below the limit of quantitation (<1.25 copies/mL) by ddPCR. (B) Plasma IgG concentrations (absorbance units) in children with MIS-C (N=15), with acute COVID-19 (N=10), and unconfirmed (N=12). Values at zero denote those that are below the limit of detection (<1.23 absorbance units) of the Euroimmun ELISA.

Figure 2A-C. Cytokine differences between those with acute COVID-19 and those with MIS-C. (A) Seventeen statistically significant cytokine/chemokine differences between children with acute COVID-19 and with MIS-C. Testing was performed on admitted pediatric patients with MIS-C (N=10) and with acute COVID-19 (N=9). Median days to sample collection was 1 day after admission in both groups (p=0.898). All values are picogram per milliliter (pg/mL). (B-C) Correlations in cytokine/chemokine profiles with

MIS-C status and associations with age and sex. All fourteen cytokines/chemokines displayed had statistically significant correlation with MIS-C status, independent of age or sex (FDR<0.05, p<0.05). (B) Cytokines/chemokines with significant correlation with age, independent of disease status, are denoted by a dashed line (FDR<0.25, p<0.05). (C) Cytokines/chemokines with significant correlation with sex, independent of disease status, are denoted by an open triangle (Δ) (FDR<0.25, p<0.05). Cytokines/chemokines which showed a notable association with disease status and sex, together, are denoted with a red star (★) (FDR>0.25, p<0.05)

Tables:

Table 1. Demographic, clinical, virologic and immunologic characteristics of children with acute COVID-19 or with MIS-C.

	No. (%)			
	Total Cohort with confirmed COVID-19, N=32	**Participants with MIS-C, N=16**	**Participants with acute COVID-19, N=16**	**p-value**
	Demographics			
Median Age, years (IQR)	7.4 (1.6-13.9)	8.7 (5.5-13.9)	2.2 (1.1-10.5)	0.18
Race				0.56
Black	8 (25)	3 (19)	5 (31)	
White	6 (19)	2 (13)	4 (25)	
Asian	2 (6)	1 (6)	1 (6)	
Other	16 (50)	10 (63)	6 (38)	
Hispanic Ethnicity	16 (50)	10 (63)	6 (37)	0.29
Male Sex	16 (50)	9 (56)	7 (44)	0.72
	SARS-CoV-2 Inclusion Criteria			
Positive SARS-CoV-2 NAT	20 (63)	8 (50)	12 (75)	0.27

Viral load, copies/mL (N1)	563.2(0-1998353.95)	63848.25(461.38-1254399.25)	307.1(0-3546348.75)	0.656
Viral load, copies/mL (N2)	736.3(0-2019419.8)	64281.3(520.12-1287013.95)	325.6(0-3447867.2)	0.656
Serology				
IgG positive	19 (59)	12 (75)	7 (44)	0.026
Not done	6 (19)	0 (0)	6 (38)	
Median IgG titer, units (IQR)	5.58 (0.28-9.71)	6.75 (0.84-10.88)	2.98 (0.28-5.76)	0.241
	Co-morbidities			
Chronic Conditions [a]	13 (41)	5 (31)	8 (50)	0.47
BMI (kg/m^2) ≥ 85th percentile for age and sex [10]				0.61
BMI unavailable or <2 years old	10 (31)	2 (13)	8 (50)	
Yes	17 (53)	10 (63)	7 (44)	
No	5 (16)	4 (25)	1 (66)	
Immunocompromised[b]	4 (13)	1 (6)	3 (19)	0.60
	Hospital Course			
Admitted	30 (94)	16 (100)	14 (88)	0.48
PICU admission	17 (53)	12 (75)	5 (31)	0.032
Oxygen Requirement	9 (28)	5 (31)	4 (25)	1.00
Intubated	1 (6)	1 (6)	0 (0)	1.00

Vasopressors	6 (19)	6 (38)	0 (0)	0.018
Treatment[c]	14 (44)	13 (81)	1 (6)	<0.001
IVIG	11 (34)	11 (69)	0 (0)	
Steroids	6 (19)	6 (38)	0 (0)	
Remdesivir	2 (6)	2 (13)	0 (0)	
Convalescent plasma	1 (6)	0 (0)	1 (6)	
Echocardiogram				<0.001
Abnormal	9 (28)	9 (56)	0 (0)	
Normal	6 (19)	4 (25)	2 (12)	
Not Done	17 (53)	3 (19)	14 (88%)	
	Outcome			
Median length of stay, days (IQR)	5.5 (3.0-7.8)	6.0 (3.0-8.3)	5 .0 (3.3-6.8)	0.835
Re-Admitted within 30 days	3 (9)	0 (0)	3 (19)	0.23
Death	1 (6)	1 (6)	0 (0)	1.00

Abbreviations: COVID-19, Coronavirus Induced Disease 2019; MIS-C, Multisystem Inflammatory Syndrome in Children; BMI, Body mass index; PICU, Pediatric Intensive Care Unit

[a]Chronic conditions include (patients may have had more than one condition): Participants with MIS-C: Chromosomal/genetic syndromes (N=3); Developmental delay (N=2); Cerebral palsy (N=1); Chronic lung disease of prematurity (N=1), History of prematurity (N=1); Asthma (N=1). Participants with acute COVID-19: Chromosomal/genetic syndromes (N=2); Congenital heart disease (N=2), Chronic lung disease (N=1), Prematurity (N=2);

Gastroesophageal reflux disease (N=2); Sickle Cell Disease (N=2); Asthma (N=1); Type 2 Diabetes Mellitus (N=1)

[b]Immunocompromised conditions include: Participants without MIS-C: Myelodysplastic syndrome (N=1), Hematopoietic stem cell transplantation (N=1), Liver transplant recipient (N=1)

[c]Patients may have received more than one treatment.

FIGURE 1A

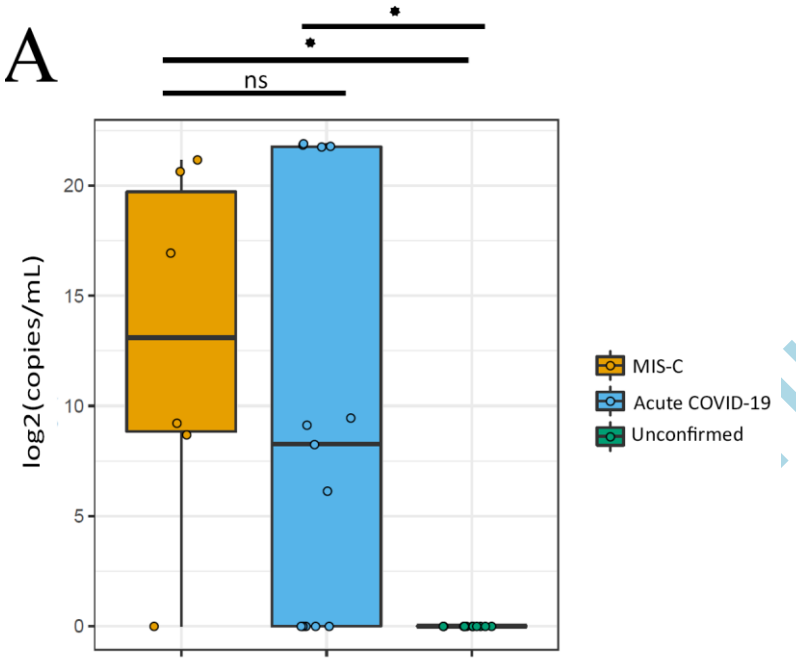

FIGURE 1B

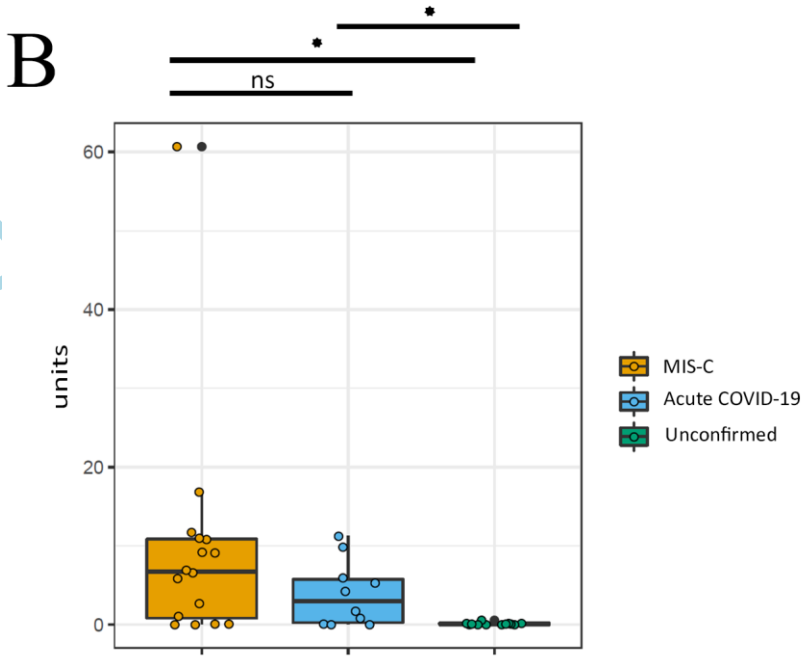

FIGURE 2A

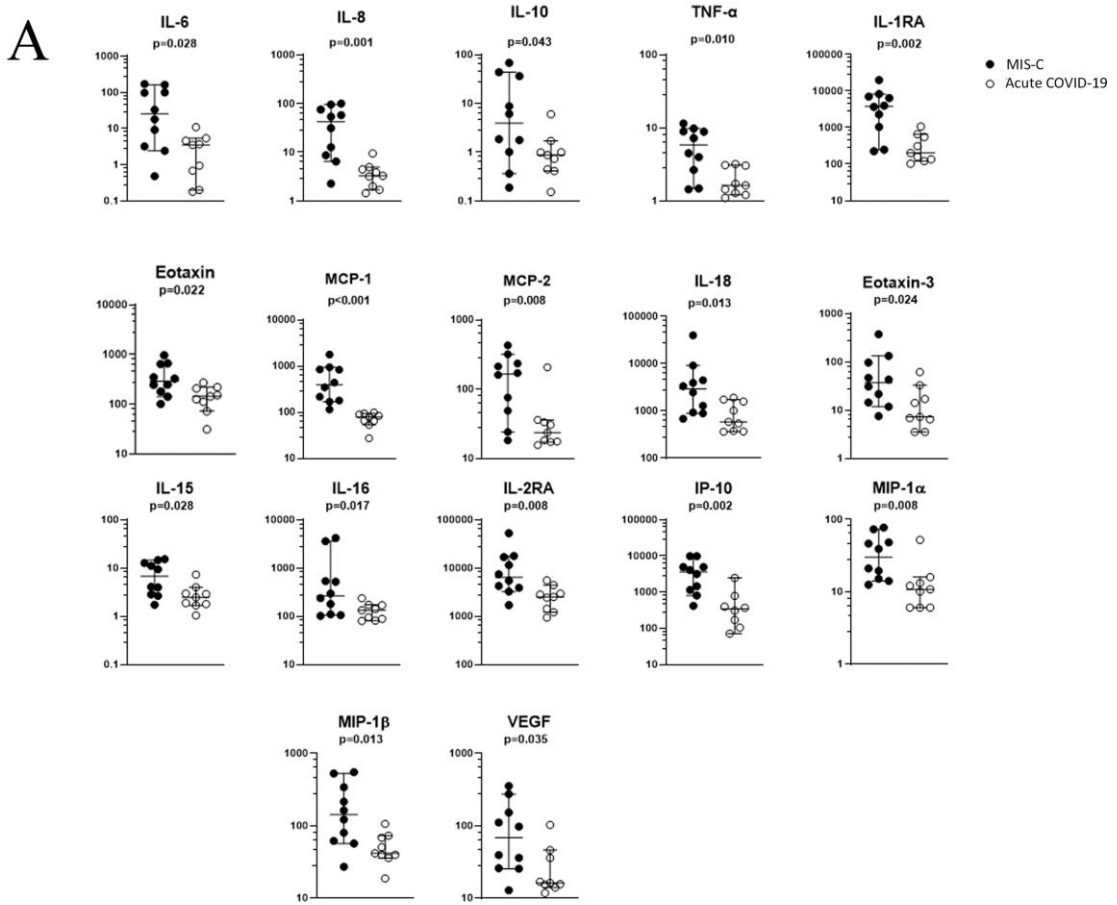

FIGURE 2B

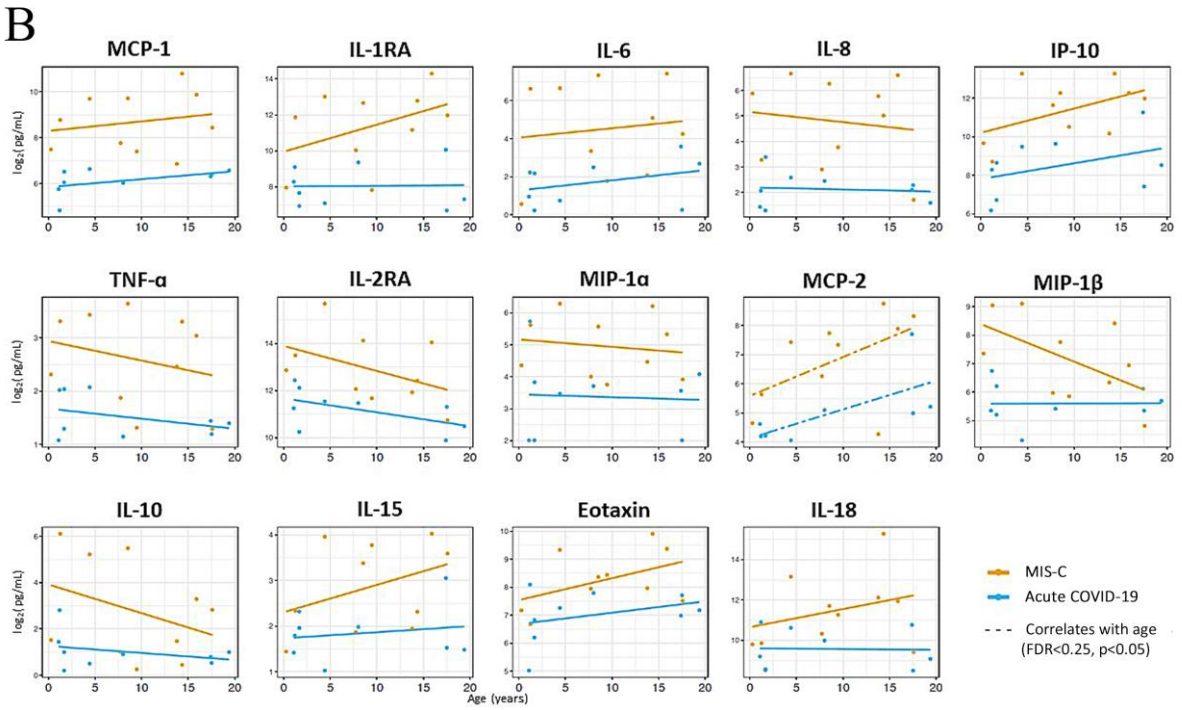

FIGURE 2C