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MAJOR ARTICLE

Serologic and Cytokine Signatures in Children With Multisystem Inflammatory Syndrome and Coronavirus Disease 2019

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Background. The serologic and cytokine responses of children hospitalized with multisystem inflammatory syndrome (MIS-C) vs coronavirus disease 2019 (COVID-19) are poorly understood.

Methods. We performed a prospective, multicenter, cross-sectional study of hospitalized children who met the Centers for Disease Control and Prevention case definition for MIS-C (n = 118), acute COVID-19 (n = 88), or contemporaneous healthy controls (n = 24). We measured severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike receptor-binding domain (RBD) immunoglobulin G (IgG) titers and cytokine concentrations in patients and performed multivariable analysis to determine cytokine signatures associated with MIS-C. We also measured nucleocapsid IgG and convalescent RBD IgG in subsets of patients.

Results. Children with MIS-C had significantly higher SARS-CoV-2 RBD IgG than children with acute COVID-19 (median, 2783 vs 146; $P < .001$), and titers correlated with nucleocapsid IgG. For patients with MIS-C, RBD IgG titers declined in convalescence (median, 2783 vs 1135; $P = .010$) in contrast to patients with COVID-19 (median, 146 vs 4795; $P < .001$). MIS-C was characterized by transient acute proinflammatory hypercytokinemia, including elevated levels of interleukin (IL) 6, IL-10, IL-17A, and interferon gamma (IFN-γ). Elevation of at least 3 of these cytokines was associated with significantly increased prevalence of prolonged hospitalization ≥8 days (prevalence ratio, 3.29 [95% CI, 1.17–9.23]).

Conclusions. MIS-C was associated with high titers of SARS-CoV-2 RBD IgG antibodies and acute hypercytokinemia with IL-6, IL-10, IL-17A, and IFN-γ.

Keywords. children; COVID-19; cytokines; MIS-C; PIMS; SARS-CoV-2; serology.

Following the onset of the coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel multisystem inflammatory syndrome in children (MIS-C) was first described in Western Europe in April 2020 [1]. This syndrome temporally follows SARS-CoV-2 infection by 2–6 weeks and is characterized by fever, systemic inflammation, multiorgan involvement, and severe disease requiring hospitalization [2]. Although the majority of patients recover without long-term sequelae [3], some develop myocardial dysfunction, shock, and respiratory failure requiring intensive care [2, 4–6]. A variety of treatment approaches have been adopted for MIS-C, which include intravenous immunoglobulin, corticosteroids, immunomodulating agents, aspirin, and anticoagulants [2, 4, 5], all of which have potential risks and uncertain benefits. Distinguishing MIS-C from alternative etiologies and identifying biomarkers of severity at the time of presentation could better inform patient management.

To date, the pathogenesis of MIS-C is poorly understood. We and others have previously reported the development of high titers of SARS-CoV-2 binding and neutralizing antibodies in patients with MIS-C [7–10]. Evidence of immune cell activation, mucosal inflammation, auto-antibody formation, and cytokine storm has also been reported [8, 11]. While previous studies have described the generalized hypercytokinemia observed in MIS-C [10, 12], the clinical correlates, predictive

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value, and time course of individual and collective cytokines are incompletely understood. In this study, we aimed to describe the distinguishing serologic and cytokine signatures associated with MIS-C diagnosis and clinical outcomes. The detailed clinical data from the cohort are the subject of a future manuscript (in review). Herein, we report the serologic and cytokine signatures of the subset of enrolled participants with MIS-C or COVID-19 who contributed blood samples for analysis.

MATERIALS AND METHODS

Patient Enrollment

This was a multicenter cross-sectional study conducted in collaboration with 4 pediatric medical centers and approved by their respective institutional review boards: Children's Healthcare of Atlanta and Emory University, Atlanta, Georgia; Phoenix Children's Hospital, Phoenix, Arizona; Arnold Palmer Hospital for Children/Orlando Health, Orlando, Florida; and Washington University, St Louis, Missouri. This activity was reviewed by the Centers for Disease Control and Prevention (CDC) and was conducted consistent with applicable federal law and CDC policy (see, eg, 45 Code of Federal Regulations [C.F.R.] part 46; 21 C.F.R. part 56; 42 United States Code [U.S.C.] §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

Hospitalized patients meeting the case definition for MIS-C or acute COVID-19 were prospectively enrolled following informed consent and assent as appropriate for age. The MIS-C case definition included any patient <21 years or age with fever, laboratory evidence of inflammation, and evidence of clinically severe illness requiring hospitalization, with multisystem organ involvement (cardiovascular, dermatologic, gastrointestinal, hematologic, neurologic, renal, or respiratory) who tested positive for SARS-CoV-2 or had recent exposure to COVID-19. MIS-C cases were adjudicated by the study sites to ensure all met the CDC case definition and that no more likely alternative diagnoses were identified during the acute hospitalization. The acute COVID-19 case definition included any patient <21 years of age with positive SARS-CoV-2 testing by any method who was hospitalized for >24 hours with no alternative diagnosis, AND had at least 2 of the following symptoms (fever, chills, rigors, myalgia, headache, sore throat, new olfactory or taste disorder) OR at least 1 of the following (cough, shortness of breath and difficulty breathing) OR severe respiratory illness with at least 1 of the following (clinical or radiographic evidence of pneumonia, acute respiratory distress syndrome [ARDS]) [13].

At the time of enrollment, blood was prospectively collected and/or residual blood was obtained from the clinical laboratories and stored at −80°C. Only samples collected prior to the administration of intravenous immunoglobulin and convalescent plasma were included, so as not to impact serological analyses. Receipt of steroids or other immunomodulatory regimens was not considered exclusionary. Participants were enrolled from 10 June 2020 until 21 April 2021 and specimens were shipped on dry ice to Emory University for analysis. A subset of patients enrolled at the Emory University site provided convalescent samples from 28 to 170 days following symptom onset. From June to October 2020, 24 contemporaneous healthy pediatric controls provided specimens following enrollment into a separate institutional review board–approved outpatient phlebotomy study at Emory.

Clinical data were abstracted from the medical record by trained abstractors using a case report form developed for MIS-C national surveillance with minor revisions. The detailed clinical data describing the cohort from the 4 hospitals will be the subject of a future manuscript (in review). The cohort herein represents the subset of patients from the clinical cohort who had MIS-C or COVID-19 and contributed blood samples for analysis.

SARS-CoV-2 Enzyme-Linked Immunosorbent Assays

The SARS-CoV-2 spike protein receptor-binding domain (RBD) protein was kindly provided by Dr Jens Wrammert [14]. RBD immunoglobulin G (IgG) enzyme-linked immunosorbent assays (ELISAs) were performed as previously described [7]. Plates were developed using o-Phenylenediamine substrate, and absorbance was read at 490 nm. Absorbance curves were generated using nonlinear regression analysis, and end-point titers were interpolated from curves by using a baseline value calculated from the pooled plasma of 8 prepandemic healthy controls. The lower limit of detection (LLOD) was 100, and undetectable titers were assigned a value of 85. ELISAs were analogously performed for SARS-CoV-2 nucleocapsid protein IgG (Sino Biological) for a subset of patients with MIS-C and acute COVID-19.

Cytokine Analysis

Serum or plasma samples were analyzed for 10 cytokines using a custom U-PLEX panel (Meso Scale Diagnostics) following the manufacturer's protocol. Cytokines on the panel included interferon gamma (IFN-γ), interleukin (IL) 1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, and tumor necrosis factor alpha (TNF-α). These cytokines were chosen based on their associations with systemic inflammation, antiviral response, COVID-19, or MIS-C in prior reports [11]. Cytokine concentrations (pg/mL) were interpolated from a standard curve. For statistical analyses, undetectable cytokine results for a given cytokine were assigned a value of 0.2 times the LLOD.

Statistical Analyses

Clinical data and laboratory results were stored in a central REDCap electronic data capture tool hosted by the CDC [15, 16]. Statistical analyses were performed using R version 4.0.2 software [17], including the packages CatPredi [18] and glmnet

[19]. Log-transformed antibody titers and cytokine concentrations were statistically compared using Fisher exact tests for categorical variables and Mann-Whitney *U* tests for continuous variables. Pearson correlation coefficients were calculated where applicable, using log-transformed serology titer and cytokine values.

To identify a cytokine signature that distinguishes MIS-C from COVID-19, we performed multivariable analysis of dichotomized cytokine concentrations using least absolute shrinkage and selection operator (LASSO) for variable selection [19]. Cytokine levels were dichotomized as elevated or not elevated using an algorithm that identified cutpoints that maximized discriminatory power [20]. LASSO was utilized to identify the 4 cytokine measurements that had the strongest combined ability to discriminate between MIS-C and COVID-19. Cytokines identified as being strong indicators of MIS-C were additionally assessed for associations with clinical findings and outcome metrics within patients with MIS-C.

RESULTS

Baseline Characteristics of Enrolled Cohort

Patients were prospectively enrolled and samples collected from 20 June 2020 to 16 April 2021, including 118 patients with MIS-C, 88 with acute COVID-19, and 24 healthy controls (Supplementary Table 1). One patient with MIS-C did not have an RBD IgG titer measurement, and another patient did not have cytokine levels tested; RBD IgG titers and cytokine levels were available for all other patients. Patients with MIS-C had a median age of 10 (interquartile range [IQR], 6–14) years, and 38.1% were female; 43.4% were non-Hispanic Black, 34.5% non-Hispanic White, 19.5% Hispanic ethnicity, and 2.7% Asian. Patients with COVID-19 had a median age of 14 (IQR, 3–17) years, and 62.5% were female; 35.3% were non-Hispanic Black, 29.4% non-Hispanic White, 34.1% Hispanic ethnicity, and 1.2% Asian. Healthy controls had a median age of 8 (IQR, 6–12) years, and 54.2% were female; 79.1% were non-Hispanic Black, 4.2% were non-Hispanic White, 8.3% Hispanic ethnicity, and 8.3% identified as other race. Almost half of MIS-C patients (48.7%) reported a preceding COVID-19–like illness a median of 21 days prior to MIS-C onset. Selected laboratory results and clinical outcome metrics are shown in Supplementary Table 1. Compared to patients with acute COVID-19, patients with MIS-C had significantly higher peak C-reactive protein, D-dimer, ferritin, brain natriuretic protein (BNP), proBNP, and troponin levels, and significantly lower nadirs of platelet count and absolute lymphocyte count, which are consistent with our previous data [7]. Among our cohort, patients with MIS-C were also significantly more likely to require vasopressors and to have an adverse cardiac outcome (defined as decreased cardiac function, myocarditis, pericardial effusion, mitral regurgitation, or coronary artery dilatation or aneurysm) compared to patients with COVID-19. The median duration of hospitalization was 5 (IQR, 4–7) days for patients with MIS-C, 63.6% required intensive care, and all survived.

Serologic Analyses

The majority of patients with MIS-C (98.3%) had elevated SARS-CoV-2 RBD IgG titers. Patients with MIS-C had significantly higher RBD IgG titers than patients with acute COVID-19 (median endpoint titer, 2783 vs 146; $P < .001$) or healthy controls (median, 2783 vs 85; $P < .001$), although patients with COVID-19 had a wide range of titers (Figure 1A, Supplementary Table 2). None of the contemporaneous healthy pediatric controls had detectable RBD IgG antibodies. Nucleocapsid protein IgG antibodies were measured for a subset of patients with acute MIS-C (n = 13) and acute COVID-19 (n = 14), and these titers correlated strongly with RBD IgG titers (Pearson $R = 0.89$ for log-transformed titer values in MIS-C patients, $P < .001$; $R = 0.66$ in COVID-19 patients, $P = .010$) (Figure 1B). Among patients with acute MIS-C, those aged 0–5 years and 6–12 years had significantly higher SARS-CoV-2 RBD IgG titers than patients 13–20 years of age ($P < .001$); however, RBD titers did not correlate with patient sex, race, or ethnicity (Supplementary Figure 1).

Convalescent samples were available for analysis from a subset of patients with MIS-C (n = 13) and COVID-19 (n = 14). For patients with MIS-C, RBD IgG declined from the acute stage to early convalescence (median, 2783 to 1135, $P = .010$; median follow-up, 50 [IQR, 41–58] days) (Supplementary Table 2). In contrast, patients with COVID-19 had significant increases in titer from the acute to the convalescent stage (median, 146–4795, $P < .001$; median follow-up, 42 [IQR, 37–52] days) (Figure 1C). SARS-CoV-2 RBD IgG titers correlated with the duration of time post–symptom onset in patients with acute COVID-19 ($R = 0.38$ for log titers, $P < .001$) (Figure 1D), in contrast to patients with MIS-C for whom it did not correlate with time. For convalescent MIS-C samples, RBD IgG titers trended toward decreasing with longer duration of time post–symptom onset ($R = -0.56$, $P = .048$) (Supplementary Figure 2).

Cytokine Analyses

All cytokines in the 10-plex panel were significantly elevated in patients with MIS-C compared to healthy controls and to patients with acute COVID-19 (Figure 2), with the exception of IL-13 (Supplementary Table 2). In convalescence, cytokines for both MIS-C and acute COVID-19 normalized to approximately the level of healthy controls (Figure 2).

The 4-variable model produced through LASSO identified the following cytokine measurements as best in differentiating MIS-C from acute COVID-19: IL-6 >25 pg/mL, IL-10 >10 pg/mL, IL-17A >4 pg/mL, and IFN-γ >250 pg/mL (Supplementary Figure 3). For MIS-C patients, 54% had elevated levels (ie, values above the aforementioned thresholds) for at least 3 of the

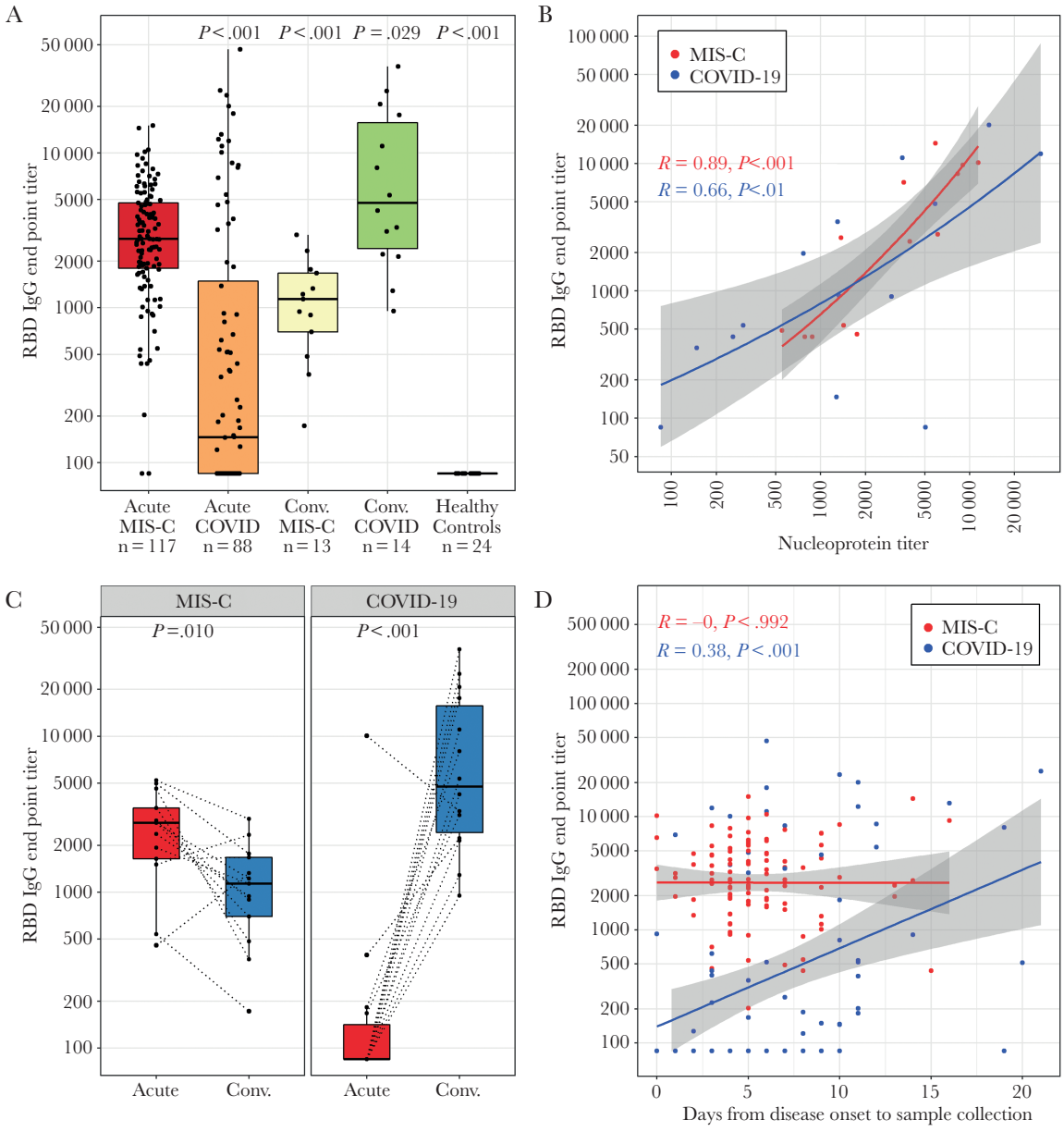


Figure 1. *A*, Severe acute respiratory syndrome coronavirus 2 immunoglobulin G (IgG) antibody profiles of patients with acute multisystem inflammatory syndrome in children (MIS-C), acute coronavirus disease 2019 (COVID-19), convalescent MIS-C, convalescent COVID-19, and healthy pediatric controls. *P* values represent comparisons between each group and acute MIS-C. *B*, Associations between receptor-binding domain (RBD) and nucleocapsid protein IgG antibody titers for patients with MIS-C (red) and COVID-19 (blue). *C*, Paired acute vs convalescent RBD IgG antibody titers among a subset of patients with MIS-C and COVID-19. *D*, Acute RBD IgG endpoint titers vs days from symptom onset among patients with MIS-C (red) and COVID-19 (blue). Associations between continuous variables are shown as Pearson correlations with log-transformed titer values. Abbreviations: Conv., convalescent; COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; MIS-C, multisystem inflammatory syndrome in children; RBD, receptor-binding domain.

4 cytokines, with 32% having elevated levels for all 4 cytokines. Conversely, only 1% of COVID-19 patients had elevated levels of at least 3 cytokines, and no COVID-19 patients had elevated levels of all 4 cytokines (Figure 3). Two-thirds of COVID-19 patients (67%) did not have elevated levels of any of these 4 cytokines. Similarly, none of the healthy controls had elevated levels of any of these 4 cytokines.

We performed stratified analysis among patients with MIS-C to determine the association between this cytokine signature with categorical and continuous clinical outcome metrics. Compared to patients with elevated levels (IL-6 >25 pg/mL, IL-10 >10 pg/mL, IL-17A >4 pg/mL, or IFN-γ >250 pg/mL) for 2 or fewer cytokines, patients with elevated levels of at least 3 of the 4 cytokines were significantly more likely to have prolonged hospitalization ≥8 days (25.8% vs 7.8%; prevalence ratio [PR], 3.29 [95% confidence interval {CI}, 1.17–9.23]), and trended toward increased prevalence of pneumonia (15.4% vs 3.8%; PR, 4.00 [95% CI, .92–17.46]) (Table 1). There was no association

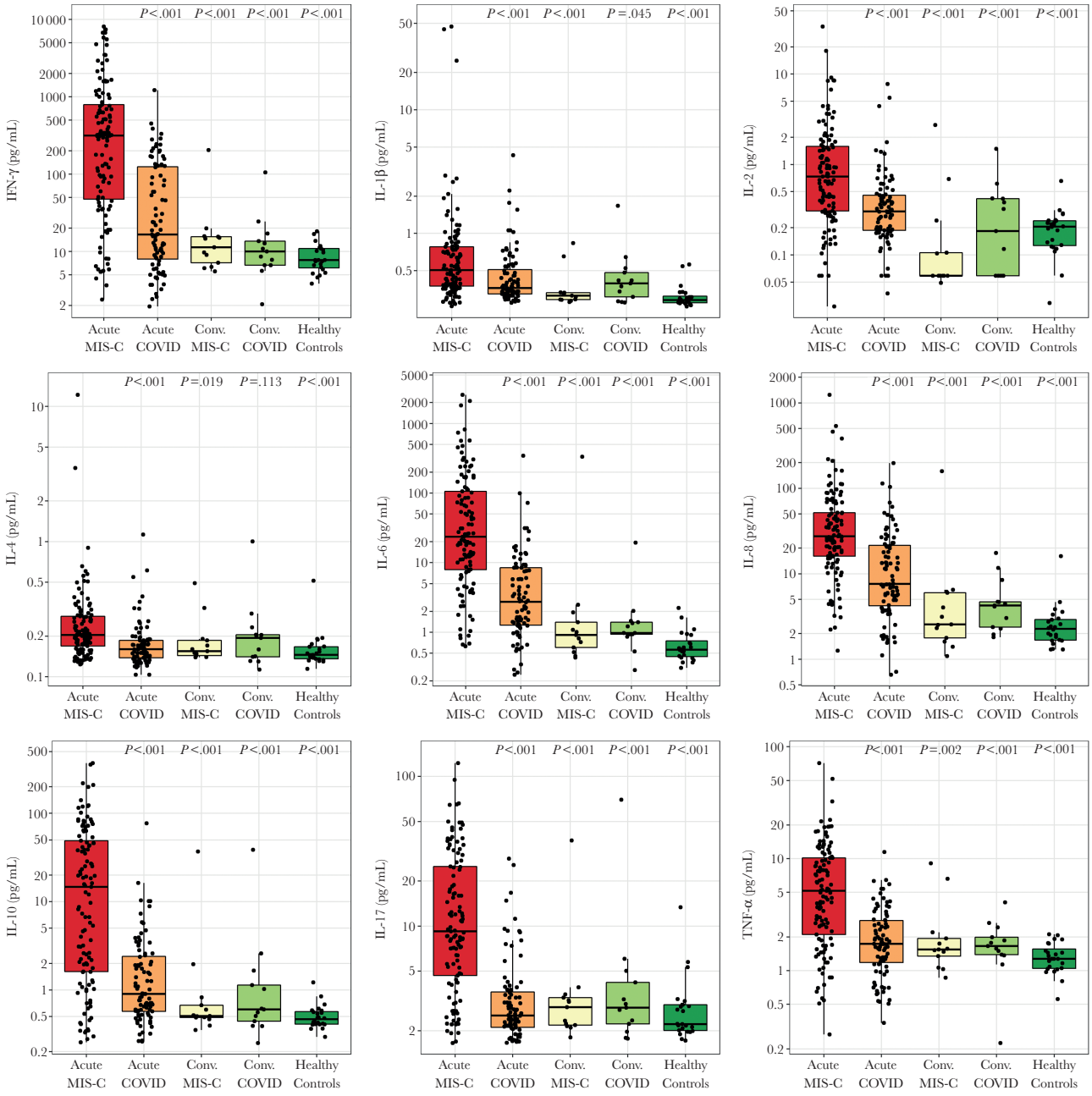
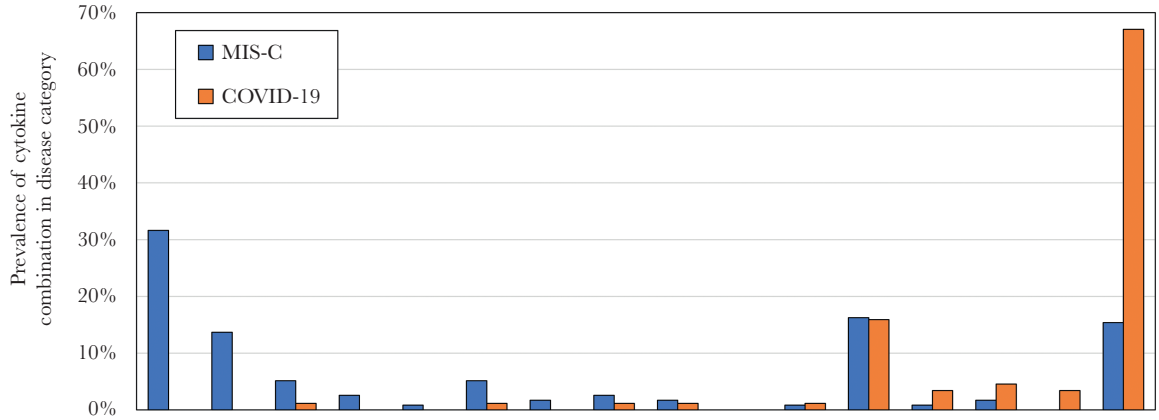


Figure 2. Cytokine profiles of patients with acute and convalescent multisystem inflammatory syndrome in children (MIS-C) and coronavirus disease 2019 (COVID-19) and healthy pediatric controls. Units = pg/mL. Data represent patients with acute MIS-C (n = 117), acute COVID-19 (n = 88), convalescent MIS-C (n = 13), convalescent COVID-19 (n = 13), and healthy controls (n = 24). Statistical comparisons of log-transformed cytokine concentrations were made using Mann-Whitney *U* tests. *P* values represent comparisons between each group and acute MIS-C. Abbreviations: COVID, coronavirus disease 2019; IFN-γ, interferon gamma; IL, interleukin; MIS-C, multisystem inflammatory syndrome in children; TNF-α, tumor necrosis factor alpha.

between having at least 3 of 4 cytokines elevated and intensive care unit admission, decreased cardiac function, any severe cardiac outcome, shock, or specific organ involvement. Similarly, no single cytokine was predictive of these outcomes. Patients with elevated levels of at least 3 of the 4 cytokines did have significantly increased peak D-dimer compared to those with ≤2 elevated cytokine levels (median, 2.81 [95% CI, 1.76–4.52] mg/L vs 2.07 [95% CI, .89–3.16] mg/L; $P = .007$) and decreased platelet nadir (median, 130×10^3 [95% CI, 93–154] cells/μL vs 173 [95% CI, 116–254] cells/μL; $P = .003$). Thus, this cytokine signature was associated with MIS-C diagnosis and some metrics of disease severity.

DISCUSSION

In this prospective, multicenter, cross-sectional study, we identified serologic and cytokine signatures of MIS-C, which



IFN-γ >250	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
IL-6 >25	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE
IL-10 >10	TRUE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	FALSE	TRUE	FALSE
IL-17A >4	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE
% MIS-C	32%	14%	5%	3%	1%	5%	2%	3%	2%	0%	1%	16%	1%	2%	0%	15%
% COVID-19	0%	0%	1%	0%	0%	1%	0%	1%	1%	0%	1%	16%	3%	5%	3%	67%

Figure 3. Cytokine signatures associated with multisystem inflammatory syndrome in children (MIS-C). Each column in the table shows a combination of cytokine levels (pg/mL), describing which cytokine thresholds are met; the bars above each table column shows the proportion of patients with MIS-C (blue) or coronavirus disease 2019 (COVID-19) (orange) with that combination of cytokine levels. For example, in the leftmost table column, all 4 of the cytokine levels have elevated = TRUE; this combination is seen in 32% of MIS-C patients and 0% of COVID-19 patients. Abbreviations: COVID-19, coronavirus disease 2019; IFN-γ, interferon gamma; IL, interleukin; MIS-C, multisystem inflammatory syndrome in children.

were hallmarked by high titers of SARS-CoV-2 RBD IgG antibodies and elevations in IL-6, IL-10, IL-17A, and IFN-γ. Overall, 98.3% of patients with acute MIS-C in our cohort had elevated SARS-CoV-2 RBD IgG antibodies, consistent with previously published results in smaller single-center cohorts [7, 9]. Patients with acute MIS-C had significantly higher SARS-CoV-2 RBD IgG titers than patients with acute COVID-19, who experienced a broad range of titers that

Table 1. Differences in Clinical Findings by Number of Elevated Cytokine Levels for Interleukin (IL) 6, IL-10, IL-17A, and Interferon-γ Among Patients With Multisystem Inflammatory Syndrome in Children

Variable	3–4 Cytokine Levels Elevated[a] (n = 65)	≤2 Cytokine Levels Elevated (n = 52)	Statistic
	No. (%)	No. (%)	PR (95% CI)
ICU admission	45 (69.2)	29 (55.8)	1.24 (.93–1.66)
Decreased cardiac function	24 (36.9)	20 (38.5)	0.96 (.60–1.53)
Any severe cardiac outcome[b]	46 (70.8)	35 (67.3)	1.05 (.82–1.34)
Shock	33 (50.8)	22 (42.3)	1.20 (.81–1.79)
Pneumonia	10 (15.4)	2 (3.8)	4.00 (.92–17.46)
Hospital length of stay ≥8 days	16 (25.8)	4 (7.8)	3.29 (1.17–9.23)
	Median (IQR)	Median (IQR)	*P* Value
ICU length of stay (days)	5 (3–6)	4 (3–5)	.200
Fibrinogen, peak (mg/dL)	566 (477–658)	613 (516–661)	.293
D-dimer, peak (mg/L)	2.81 (1.76–4.52)	2.07 (0.89–3.16)	.007
Troponin, peak (ng/mL)	0.1 (0.03–0.41)	0.05 (0.02–0.19)	.230
BNP, peak (pg/mL)	834 (361–2499)	340 (172–1036)	.139
proBNP, peak (ng/L)	5026 (1700–11 290)	2600 (1226–13 871)	.554
CRP, peak (mg/dL)	18 (12–26)	16 (11–20)	.122
Ferritin, peak (ng/mL)	538 (396–1147)	436 (224–1087)	.163
Platelets, nadir (10^3 cells/μL)	130 (93–154)	173 (116–254)	.003
Lymphocytes, nadir (cells/μL)	600 (400–1191)	877 (492–1300)	.260

Categorical and continuous outcome metrics were compared among pediatric patients with multisystem inflammatory syndrome who had 3–4 cytokines elevated vs those who had ≤2 cytokines elevated.

Abbreviations: BNP, brain natriuretic peptide; CI, confidence interval; CRP, C-reactive protein; ICU, intensive care unit; IQR, interquartile range; proBNP, pro-brain natriuretic peptide; PR, prevalence ratio.

[a]Cytokine cutoffs for this analysis: interleukin (IL) 6, >25 pg/mL; IL-10, >10 pg/mL; IL-17A, >4 pg/mL; and interferon-γ, >250 pg/mL.

[b] Defined as 1 or more of the following: decreased cardiac function, myocarditis, pericardial effusion, mitral regurgitation, and coronary artery dilatation or aneurysm.

correlated with time from symptom onset. Interestingly, children with acute MIS-C aged 0–5 and 6–12 years had significantly higher SARS-CoV-2 RBD IgG titers than adolescent patients 13–20 years of age. One possible explanation of this finding is that older children with MIS-C may be more likely to present concurrently with acute COVID-19, prior to their development of high-titer SARS-CoV-2 antibodies [2]. In contrast to patients with acute COVID-19, patients with MIS-C had significant declines in SARS-CoV-2 RBD IgG titers during early convalescence. This may be primarily attributed to the timing of acute MIS-C following SARS-CoV-2 infection, which typically follows peak COVID-19 transmission in the community by 2–6 weeks [4, 21, 22]. Nevertheless, this natural waning of RBD immune response may inform the optimal timing of vaccination post–MIS-C, as susceptibility to reinfection likely increases with time following the initial SARS-CoV-2 infection.

In addition to having high titers of SARS-CoV-2 IgG antibodies, patients with MIS-C had significant elevations of multiple proinflammatory and Th1-type cytokines, consistent with a cytokine storm. Although the term cytokine storm lacks a strict definition, it has been described as the dysregulated release of interleukins, interferons, tumor necrosis factors, and other small-molecule mediators that results in broad immune cell activation and an end-organ damage [23]. Hypercytokinemia, and specifically elevations in IL-6, IL-8, and TNF-α, has been associated with severe outcomes and death among adult patients with COVID-19 [24, 25]. However, less is known about the cytokine signatures of pediatric COVID-19 and MIS-C. In this study, we found that IL-6, IL-10, IL-17A, and IFN-γ were strongly associated with the diagnosis of MIS-C. These results overlap with the findings of previously published reports of cytokine analyses in smaller cohorts of patients with MIS-C. For example, Consiglio et al found that IL-6 and CXCL10 contributed to the cytokine storm observed in patients with MIS-C [11]. Diorio et al found that the most discriminatory cytokines among a small cohort of patients with MIS-C and severe COVID-19 were IL-10 and TNF-α [26]. Moreover, Gruber et al found IL-6 and IL-17A to be elevated in MIS-C, in addition to other cytokines involved in inflammation, lymphocytic and myeloid cell chemotaxis and activation, and mucosal immune dysregulation [8].

The cytokine responses we observed in patients with MIS-C were distinct from acute and convalescent COVID-19. The unique serologic and cytokine signatures we identified could add diagnostic and prognostic value for patients presenting with signs and symptoms compatible with MIS-C. The diagnosis of MIS-C in the United States is currently based upon meeting the CDC case definition, which requires the presence of fever, multiorgan involvement, systemic inflammation, lack of an alternative explanatory diagnosis, and epidemiologic link or positive test for SARS-CoV-2. However, clinical features overlap with other inflammatory syndromes and there is concern that the increasing SARS-CoV-2 seroprevalence, both due to natural infection and vaccination, may confound the interpretation of spike or RBD SARS-CoV-2 antibody titers in cases of suspected MIS-C. In our cohort, we found that almost all patients with MIS-C had high titers of RBD IgG antibodies, and that RBD titers correlated with nucleocapsid IgG titers with the log-titer levels having a strong linear association. Thus, quantitative nucleocapsid serology is likely to retain applicability as a biomarker of MIS-C in the setting of widespread pediatric immunization with spike protein–based vaccines. Nevertheless, few prognostic indicators have been identified for either short- or long-term MIS-C outcomes. In our study, we found that IL-6, IL-10, IL-17A, and IFN-γ were significantly associated with prolonged duration of hospitalization, but not other categorical outcomes. Elevations in at least 3 of the 4 cytokines was also associated with elevated peak D-dimer and decreased platelet nadir, both of which are thought to contribute to MIS-C pathophysiology. All cytokine levels returned to normal within approximately 2 months, suggesting that MIS-C represents a transient state of immune activation and hyperinflammation following SARS-CoV-2 infection.

Understanding the pathogenesis of MIS-C could better inform targeted approaches to patient management. Current treatment strategies commonly include intravenous immunoglobulin, systemic corticosteroids, or immunomodulatory monoclonal antibodies, such as the IL-1β inhibitor anakinra. All of these interventions target broad or specific components of the inflammatory cascade, to reduce the risk that the transient state of immune activation leads to end-organ damage. However, the underlying trigger for the systemic hyperinflammatory response remains elusive. A recent study demonstrated that persistence of SARS-CoV-2 in the gastrointestinal tracts of patients with MIS-C was associated with a breakdown of mucosal integrity and subsequent spike protein antigenemia and hyperinflammation [27]. While this explanation could correspond with the prominent gastrointestinal symptoms observed in MIS-C, the finding of SARS-CoV-2 antigenemia has not been universal [28]. The reasons why antigenemia may trigger delayed but not acute systemic hyperinflammation are similarly unclear. Interestingly, Kumar et al found significantly elevated markers of microbial translocation in children with MIS-C, which could contribute to systemic inflammatory responses [29]. Future studies are needed to elucidate MIS-C pathogenesis and to prospectively evaluate the efficacy of various treatment modalities upon MIS-C outcomes.

Strengths of our study include the prospective, multicenter design and large sample size of well-characterized patients with MIS-C. Limitations include the small numbers of convalescent samples available and the lack of long-term follow-up. The inclusion criteria of our study may have limited enrollment of clinically ambiguous cases that did not meet the prespecified case definitions. We did not assess serologic and cytokine

profiles of other pediatric hyperinflammatory conditions, such as Kawasaki disease or toxic shock syndrome. From a diagnostic standpoint, the comparison of immune profiles with acute COVID-19 may have greatest applicability to adolescents and/or adults with MIS-A, who have a more heterogeneous clinical presentation that often overlaps with acute COVID-19 [30, 31]. We were unable to evaluate treatment effects on serologic and cytokine profiles and clinical outcomes, although there were no pediatric deaths due to MIS-C and available convalescent samples showed cytokine normalization. We did not measure neutralizing antibody responses, although we and others have previously shown that these correlate with SARS-CoV-2 RBD IgG binding antibodies [7, 32]. Similarly, we were unable to evaluate associations between serologic and cytokine profiles with nasopharyngeal viral load by reverse-transcription polymerase chain reaction. Although the vaccination status of the children was not prospectively collected, all enrollments preceded the US Food and Drug Administration emergency use authorization of COVID-19 vaccination for children <16 years of age. Enrollments also preceded the surges of SARS-CoV-2 Delta and Omicron variants in the United States. The effects of emerging SARS-CoV-2 variants of concern on MIS-C disease pathogenesis and clinical responses remain areas of active research.

In conclusion, MIS-C was associated with high titers of SARS-CoV-2 RBD IgG antibodies and elevated IL-6, IL-10, IL-17A, and IFN-γ. In convalescence, antibody titers waned and hypercytokinemia resolved, suggesting transient immune activation and hyperinflammation in acute MIS-C.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Patient consent. All human subjects were prospectively enrolled following written or verbal informed consent and assent as appropriate for age and as approved by each site's local institutional review board. Verbal consent and assent were allowed at some sites for the purpose of limiting exposure to severe acute respiratory syndrome coronavirus 2. All procedures followed were in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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