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Pediatric SARS-CoV-2: Clinical Presentation, Infectivity, and Immune Responses

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Abstract

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Data sharing: The data obtained as part of this study are available from the corresponding author upon reasonable request.

Objectives: As schools plan for re-opening, understanding the potential role children play in the coronavirus infectious disease 2019 (COVID-19) pandemic and the factors that drive severe illness in children is critical.

Study design: Children ages 0-22 years with suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection presenting to urgent care clinics or being hospitalized for confirmed/suspected SARS-CoV-2 infection or multisystem inflammatory syndrome in children (MIS-C) at Massachusetts General Hospital (MGH) were offered enrollment in the MGH Pediatric COVID-19 Biorepository. Enrolled children provided nasopharyngeal, oropharyngeal, and/or blood specimens. SARS-CoV-2 viral load, ACE2 RNA levels, and serology for SARS-CoV-2 were quantified.

Results: A total of 192 children (mean age 10.2 +/− 7 years) were enrolled. Forty-nine children (26%) were diagnosed with acute SARS-CoV-2 infection; an additional 18 children (9%) met criteria for MIS-C. Only 25 (51%) of children with acute SARS-CoV-2 infection presented with fever; symptoms of SARS-CoV-2 infection, if present, were non-specific. Nasopharyngeal viral load was highest in children in the first 2 days of symptoms, significantly higher than hospitalized adults with severe disease ($P = .002$). Age did not impact viral load, but younger children had lower ACE2 expression (P=0.004). IgM and IgG to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein were increased in severe MIS-C (P<0.001), with dysregulated humoral responses observed.

Conclusion: This study reveals that children may be a potential source of contagion in the SARS-CoV-2 pandemic in spite of milder disease or lack of symptoms, and immune dysregulation is implicated in severe post-infectious MIS-C.

As schools plan for re-opening, debates around the role children play in the COVID-19 pandemic persist. Concerns have been raised as to whether allowing children to congregate in the classroom will fuel the spread of the pandemic. On an individual level, families are worried how SARS-CoV-2 infection could affect their children and family. Particular concern is elevated for families belonging to low socio-economic classes, where the prevalence of SARS-CoV-2 infection is higher, and where multi-generational co-habitation is the norm, increasing the risk of transmitting the infection to vulnerable grandparents and older adults(1).

The manner in which children contribute to the spread of SARS-CoV-2 is unclear. Children are less likely to become seriously ill from SARS-CoV-2(2); however, asymptomatic carriers, including children, can spread infection and carry virus into their household.[3] Children infected with SARS-CoV-2 tend to have milder symptoms with significantly lower mortality than is seen in adult infection(4). It has been hypothesized that children have reduced incidence of COVID-19 because ACE2 expression in the nasopharynx increases with age(5); however ACE2 expression has not been studied in the upper airways of children infected with SARS-CoV-2. Understanding infectious burden and potential for transmissibility within the pediatric population is critical for developing both short- and long-term responses, including public health policies, to the current pandemic.

Although an acute SARS-CoV-2 infection tends to be mild or symptom-free in most pediatric cases, some children develop a multisystem inflammatory syndrome (MIS-C)(6, 7)

several weeks after possible SARS-CoV-2 infection or exposure, with severe cardiac complications, including hypotension, shock, and acute heart failure(8). Understanding post-infectious immune responses in pediatric SARS-CoV-2 infection(9), especially MIS-C, is critical for designing treatment and prevention strategies.

Here, we describe the pediatric impact of COVID-19, specifically focusing on viral burden, susceptibility to disease, and immune responses.

Methods

Patient selection:

Pediatric patients ≤22 years of age presenting to Massachusetts General Hospital Respiratory Infection Control clinics for medical evaluation of symptoms concerning for COVID-19 or admitted for acute symptoms related to COVID-19 or MIS-C were offered enrollment in the Institutional Review Board (IRB)-approved MGH Pediatric COVID-19 Biorepository (#2020P000955). For the ACE2 gene expression analysis, children presenting for well visits and newborns born during the COVID-19 pandemic were enrolled in the MGH Pediatric COVID-19 Biorepository. For the virology and antibody studies, adult patients being evaluated for COVID-19 in the outpatient or inpatient setting were enrolled through the IRB-approved MGH COVID-19 Biorepository (#2020P000804) (Table 1; available at www.jpeds.com).

Once informed consent, and if appropriate, assent, were verbally obtained by the patients or parent/guardian in accordance with IRB guidelines, nasopharyngeal and oropharyngeal swabs were obtained and placed in phosphate buffered saline. The samples were immediately aliquoted and stored at −80°C. Venipuncture was performed; plasma and serum were collected and immediately stored at −80°C.

Study definitions:

SARS-CoV-2 (+) individuals had a nasopharyngeal swab sample positive for SARS-CoV-2 by clinical quantitative polymerase chain reaction (qPCR) testing. SARS-CoV-2 (−) individuals had negative nasopharyngeal qPCR testing. MIS-C was defined per the Centers for Disease Control and Prevention (CDC) criteria: fever >38°C for >24 hours, laboratory evidence of inflammation, at least two organs involved, and no alternative plausible diagnoses and a positive SARS-CoV-2 test by RT-PCR, serology or antigen test, or exposure to an individual. with COVID-19 within 4 weeks prior to the onset of symptoms.

Data collection:

Medical records were reviewed to assess demographic and clinical factors, including age, medical history, presenting features and clinical testing, household contacts, and other possible risk factors at presentation. Data were stored in a REDcap database.

SARS-CoV-2 viral load quantification:

SARS-CoV-2 RNA levels were quantified with a quantitative viral load assay using the US CDC 2019-nCoV_N1 primers and probe set as previously described(10). Plasma and

respiratory samples were centrifuged at approximately 21,000 x g for 2 hours at 4°C. RNA was extracted from serum and respiratory specimens using the TRIzol-LS (Thermo Fisher Scientific Inc, Waltham, MA, USA)-based method, followed by RNA purification, and quantification with the 1X TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher). Quantification of the Importin-8 (IPO8) housekeeping gene RNA level was performed to determine the quality of the respiratory sample collection(11-13). An internal virion control (RCAS) was spiked into each sample and quantified to determine the efficiency of RNA extraction and qPCR amplification.(14) SARS-CoV-2 pseudoviral reference standards (SeraCare, Milford, MA, USA) were used as positive controls for each run. SARS-CoV-2 viral loads below 40 RNA copies/mL were categorized as undetectable and set at 1.0 $\log_{10}$ RNA copies/mL.

ACE2 expression in the upper airway

cDNA was transcribed from RNA extracted from nasopharyngeal and oropharyngeal swabs using TRIzol-LS reagent (Thermo Fisher) and then purified by isopropanol extraction. qPCR standards were created using a hACE2 plasmid and MEGAscript T7 transcription kit (Thermo Fisher), purified with the RNeasy MinElute spin column kit (Qiagen, the Netherlands), and quantified by nanodrop. ACE2 and IPO8 Gene expression was assessed by qPCR using iTaq Universal SYBR Green mix (Bio-Rad Laboratories, Hercules, CA, USA) with ACE2 primers (FWD AAACATACTGTGACCCCGCAT, REV CCAAGCCTCAGCATATTGAACA) as previously used(15) and IPO8 primers (Bio-Rad Laboratories, Hercules, CA, USA). ACE2 and IPO8 RNA were used to generate standard curve to quantitate copy numbers per sample and ACE2 expression relative to IPO8 was calculated as previous(16).

IgG and IgM titers measured by ELISA:

SARS2-CoV2-RBD (in-house, HEK293 cells provided by Aaron Schmidt, Ragon Institute) and SARS2-CoV2-NC (Aalto Bio Reagents Ltd., Ireland) specific plasma antibodies were quantified by ELISA. The average plus 5x or 3x standard deviation of included negative adult plasma controls were defined as negative cutoff for IgG or IgM, respectively. SARS-CoV-2-RBD specific monoclonal human IgG1 or IgM antibody (clone: CR3022) was added in a two-fold dilution curve starting at 2.5ug/ml to each plate and specific IgG or IgM concentrations were calculated.

IgG1 and IgM titers measured by Luminex:

SARS2-CoV2-RBD, SARS2-CoV2-NC, SARS2- CoV2-S (provided by Eric Fischer, Dana Farber), and RBD domains of the coronavirus strains NL-63, HKU1, 229E and OC43 (in-house, provided by Aaron Schmidt) specific antibody isotypes were analyzed by Luminex multiplexing(17). Antigens were carboxy-coupled to Luminex microspheres (Luminex Corp, TX, USA) and incubated with polyclonal plasma samples containing IgM and IgG1. Isotypes were probed with fluorophore-tagged secondary antibody and relative concentrations analyzed by flow cytometry.

Statistical Analyses:

Mann-Whitney U-test assessed statistical significance between two outcomes; Kruskal-Wallis test assessed comparisons of continuous variables. For all categorical comparisons, the Fisher exact test was used. The Spearman rank correlation tested relationships between two variables. Prism software was used to analyze and graph data.

Results

The MGH Pediatric COVID-19 Biorepository enrolled 192 patients (mean age 10.2 +/− 7 years), whose demographics are summarized in Table 2 (available at www.jpeds.com). Of all enrolled, 49 (26%) were SARS-CoV-2 (+), 18 (9%) had MIS-C, and 125 (65%) were SARS-CoV-2 (−).

Patient demographics

Children ages 0-22 years participated in this study, with children ages 11-16 years most highly represented in the SARS-CoV-2 (+) cohort (16, 34%) and children ages 1-4 years most highly represented in the MIS-C cohort (7, 39%). Only 2 (4%) of the SARS-CoV-2 (+) cohort were <1 year of age, although this was previously reported as a higher risk age-group(18). Sex was equally distributed between children with and without acute SARS-CoV-2 infection, although there was a male predominance in the MIS-C group (14, 78%). Latino/Hispanic children were most highly represented in both the SARS-CoV-2 (−) and SARS-CoV-2 (+) groups. Twenty-five (51%) of children infected acutely with SARS-CoV-2 came from low-income communities, as compared with 1 (2%) from high-income communities (Fisher exact test, P<0.001).

All children enrolled in the Pediatric COVID-19 biorepository had the option of providing nasopharyngeal, oropharyngeal, and blood specimens for research. Eighty-three children provided a nasopharyngeal specimen, 105 provided an oropharyngeal specimen, and 100 provided a blood sample.

Presenting symptoms

SARS-CoV-2 infection and non-COVID-19-related illnesses presented similarly. Both SARS-CoV-2 (−) and SARS-CoV-2 (+) children commonly reported fever, (62, 40% vs 25, 51%, respectively), cough (55, 36% vs 23, 47%), congestion (29, 19% vs 17, 35%), rhinorrhea (29, 19% vs 14, 29%), and headache (33, 21% vs 13, 27%), none of which were significantly different between the two groups. Anosmia was more common in the SARS-CoV-2 (+) group (3, 2% vs 10, 20% P=<0.001), as was sore throat (26, 28% vs 17, 35%, P=0.04). In addition to fever, MIS-C presented more often with nausea/vomiting (5, 29%, P<0.001) and rash (5, 28%, P<0.001) and less often with symptoms of an upper respiratory tract infection. Temperatures documented on examination did not differ among the three cohorts (Figure 1 and Table 3 [available at www.jpeds.com]).

Co-morbidities

None of the SARS-CoV-2 (+) or MIS-C children had heart disease, hypertension, or diabetes, which are risk factors for infection in the adult population(19); however, 13 (27%)

of SARS-CoV-2 (+) children were obese, as compared with 2 (11%) of the MIS-C cohort. Asthma was a common feature in SARS-CoV-2 (−) patients (29, 19%) whereas SARS-CoV-2 (+) and MIS-C patient groups displayed typical population rates of asthma(20) (6, 12% and 2,11%, respectively). Other pulmonary diseases, immune/autoimmune diseases, and neuro/neurodevelopmental diseases were assessed and were not seen in high levels in any cohort.

Nine (18%) SARS-CoV-2 infected children and 10 (56%) children with MIS-C did not have a known infected household contact. Of the children acutely infected with SARS-CoV-2, 26 (53%) attended grade school. None of the 7 preschool/kindergarteners tested positive for SARS-CoV-2 or developed MIS-C.

SARS-CoV-2 viral load

Nasopharyngeal and oropharyngeal swabs and serum were tested to quantify SARS-CoV-2 viral load. Higher levels of viral load were detected in nasopharyngeal swabs compared with oropharyngeal swabs (unpaired t-test, P=0.01, Figure 2, A). Only 2 (11%) children with MIS-C had a detectable viral load from nasopharyngeal swabs (Figure 2, A). Viral load in respiratory secretions of children was high, despite mild or absent symptoms, at 6.2 $\log_{10}$ RNA copies/ml (range 1.0-8.9 $\log_{10}$ RNA copies/ml) during days 0-2 of symptoms. Of the 11 asymptomatic children presenting for SARS-CoV-2 testing based on exposure to an infected individual rather than symptoms, 3 (27%) tested positive for SARS-CoV-2 infection. Pediatric patients displayed no apparent difference in viral load compared with adults requiring intubation for severe SARS-CoV-2 infection when stratified by time. Viral load in children in the asymptomatic/early infection phase was significantly higher than in hospitalized adults with severe disease with over 7 days of symptoms (P=0.002) (Figure 2, B). Nasopharyngeal viral load decreased over time (Spearman r=−0.56, P=0.003) (Figure 2, C). Age did not impact the ability to carry a high viral load (Figure 2, D). Of note, our cohort included a limited number of infants, and children <6 years of age were less likely to provide a nasopharyngeal swab for research. No SARS-CoV-2 RNA was detected in the serum of any children (Figure 2, A).

SARS-CoV-2 viral binding sites

ACE2 gene expression was quantified from nasopharyngeal and oropharyngeal swabs of SARS-CoV-2(+) and SARS-CoV-2 (−) children, plus from swabs from asymptomatic children presenting for well-visits and from newborns who were also enrolled in the MGH Pediatric COVID-19 biorepository. ACE2 expression was higher in SARS-CoV-2 infected children (including MIS-C) as compared with non-infected children (P=0.004) (Figure 3, A). Within the SARS-CoV-2 infected cohort, ACE2 expression did not correlate with viral load, suggesting that although increased ACE2 expression increased susceptibility for infection, once infected, children could carry high viral loads regardless of level of ACE2 expression (Figure 3, B). Children <10 years had lower ACE2 expression as compared with older children (P=0.004) (Figure 3, C). Within the pediatric cohort, ACE2 expression increased with age (Spearman r=0.20, P=0.02) (Figure 3, D).

SARS-CoV-2 antibody response

To determine immune responses to SARS-CoV-2 infection, antibodies to the receptor binding domain (RBD) component of the spike protein of SARS-CoV-2 were quantified. Children with acute SARS-CoV-2 infection were more likely than MIS-C to have an elevated IgM to RDB (P=0.01), consistent with the resolution of acute SARS-CoV-2 infection in children with MIS-C (Figure 4, A). IgG levels increased in acute SARS-CoV-2 infection with increased duration of symptoms (Spearman correlation, r=0.44, P=0.02, Figure 4, B). Children with severe MIS-C (defined as children with MIS-C with hypotension or cardiac abnormalities requiring therapeutic intervention such as steroids, IVIG, and/or anakinra) were more likely to have elevated IgM and IgG SARS-CoV-2 responses compared with mild MIS-C (Fisher exact test, each P<0.001) (Figure 4, C). Both IgM and IgG SARS-CoV-2 levels in mild MIS-C were below a threshold of 5μg/ml (Figure 4, D), consistent with waning immune responses seen in adults following acute infection(21). There was no correlation of IgG level with duration of symptoms seen in MIS-C (Figure 4, E). Children presenting with severe MIS-C tended to have broadly elevated IgG responses to a multitude of respiratory viruses, including other coronaviruses, 229E, NL63, HKU1, and OC43, Respiratory Syncytial Virus (RSV), and influenza. This was not seen in milder cases of MIS-C, acute SARS-CoV-2 infection in children, adults hospitalized for SARS-CoV-2 infection and recovered adults, pointing to a generalized enhancement of humoral immune responses as a marker of severe MIS-C (Figure 4, F, Figure 5 [available at www.jpeds.com], and Figure 6 [available at www.jpeds.com]).

To further characterize the inflammatory response in MIS-C, correlations between SARS-CoV-2 antibodies and inflammatory markers were analyzed. These included CRP, a generalized inflammatory marker; ferritin, a marker of macrophage activation; and NT-proBNP, a peptide secreted by cardiomyocytes during heart failure (Figure 7; available at www.jpeds.com). A positive correlation was found between ferritin and both IgM SARS-CoV-2 (Spearman correlation r=0.55, P=0.03) and IgG SARS-CoV-2 (Spearman correlation r=0.42, P=0.10), suggesting an interplay between monocytes/macrophages and SARS-CoV-2 antibodies in MIS-C. A significant correlation was noted with NT-proBNP and IgG SARS-CoV-2 (Spearman correlation r=0.63, P=0.008), although there was no correlation between NT-proBNP and IgM SARS-CoV-2.

Discussion

We present findings from the largest pediatric COVID-19 biospecimen repository to date, describing viral load, ACE2 expression, and antibody responses as they relate to children with acute SARS-CoV-2 infection and MIS-C. We found that children can carry high levels of virus in their upper airways, particularly early in an acute SARS-CoV-2 infection, yet they display relatively mild or no symptoms. However, there was no age correlation with viral load, indicating that infants through young adults can carry equally high levels of virus. However, SARS-CoV-2 infected children have higher levels of ACE2 expression, which may pre-dispose certain children to infection. Children with MIS-C do not have high levels of viral load on nasopharyngeal or oropharyngeal viral testing, nor do they have detectable viremia, however, they do have hyperactive antibody responses.

From an infection-control perspective, it is critical to identify infected children early for quarantine purposes. One third of school-aged children presenting with illness during the height of the local pandemic were found to have SARS-CoV-2 infection. However, children display relatively mild or no symptoms. Although ACE2 expression was increased in SARS-CoV-2 infected children, ACE2 expression did not impact viral load within the upper airway. Similarly, although younger children had reduced ACE2 expression, age also did not impact viral load. This suggests that regardless of disease susceptibility, children can carry high viral loads, which is a key consideration when opening up schools and daycare centers.

Moreover, when present, the symptoms of SARS-CoV-2 are non-specific and overlap considerably with non-COVID-related illnesses. Identifying SARS-CoV-2 infection in children will become even more challenging during pollen allergy season and influenza season this fall. Further, some children carry very high viral loads even before symptoms develop. On the other hand, children with severe symptoms, e.g. MIS-C, do not have high levels of viral load on nasopharyngeal or oropharyngeal viral testing, nor do they have detectable viremia. Overall, the lack of correlations between viral load and symptoms will complicate infection-control strategies for children.

Children with severe MIS-C have elevated SARS-CoV-2 IgM and IgG levels; IgG levels are not only elevated in SARS-CoV-2 but also in the other coronaviruses, influenza, and RSV. The broad, nonspecific antibody response points to T and B cell over-reactivity, or to auto-antibodies that may be driving an inflammatory process causing MIS-C(22). Elevated ferritin levels in MIS-C, which positively correlate with SARS-CoV-2 serology, also suggest an interplay with macrophage activation. Further, SARS-CoV-2 IgG are positively correlated with NT-proBNP, a marker of heart failure, which could indicate mechanism of disease or provide a correlation with disease severity.

Limiting the spread of SARS-CoV-2 infections in children is of particular concern as schools plan for re-opening. Our findings suggest that it would be ineffective to rely on symptoms or temperature monitoring to identify SARS-CoV-2 infection. Instead, infection control measures should minimize the possibility of viral spread, with focus on strategies including social distancing precautions, mask use, and/or remote learning. Moreover, schools could screen all students for SARS-CoV-2 infection and establish routine screening protocols. Without infection control measures such as these, there is significant risk that the pandemic will persist, and children could carry the virus into the home, exposing adults who are at higher risk of developing severe disease. This risk is particularly high in lower income communities where household size may be larger with multi-generational co-habitation and greater housing density. These recommendations contradict previous reports from the initial phase of the pandemic, which found children to be less likely to be the index case for viral transmission within a household(23). However, in our cohort, nearly 20% of acute SARS-CoV-2 infections and over half of the MIS-C cases did not have a known household exposure to SARS-CoV-2. Although transmissibility was not assessed in this study, children with high viral loads and non-specific symptoms including rhinorrhea and cough can likely transmit SARS-CoV-2 as easily as other viral infections spread by respiratory particles. If schools were to re-open fully without necessary precautions, it is likely that children will play a larger role in this pandemic.

Our initial findings show that although a low expression of ACE2 in younger children (<10 years of age) likely corresponds to reduced infection rates, children of all ages, once infected, can carry high SARS-CoV-2 viral loads. Symptom monitoring is an ineffective strategy for identifying infected children. Children can develop severe illness during the post-infectious stage with a hyperinflammatory antibody response. Potential transmission of SARS-CoV-2 between children and families should be considered when designing strategies to mitigate the COVID-19 pandemic.

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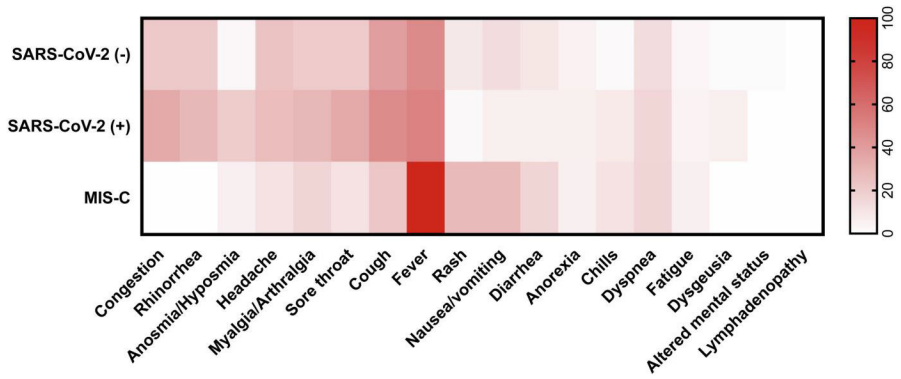

Figure 1:

Presenting symptoms of enrolled patients. Red color intensity depicts increased prevalence of a symptom within each cohort. Patients were grouped by SARS-CoV-2 qPCR results (positive or negative) or diagnosis of MIS-C.

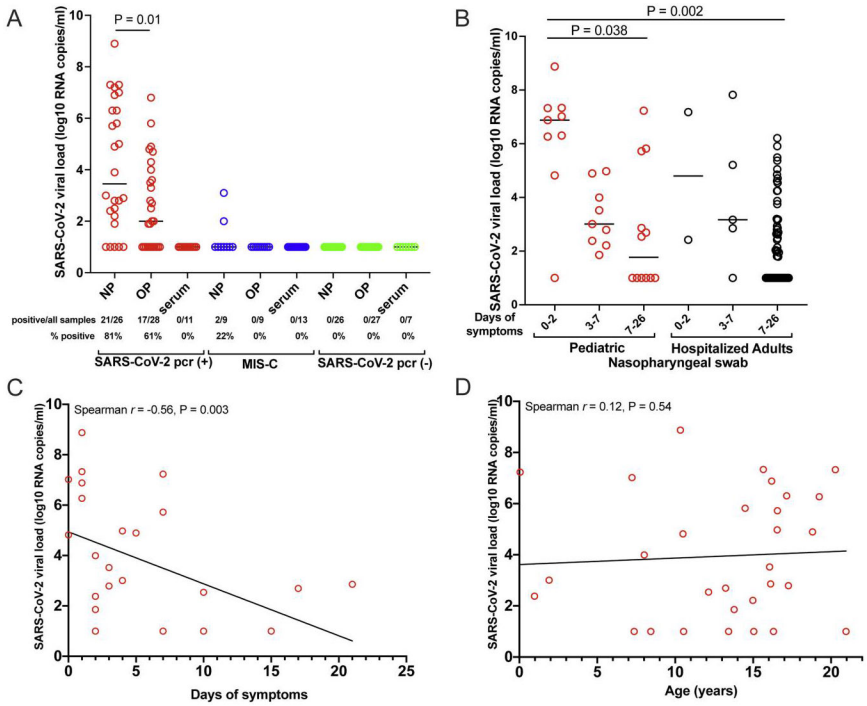

Figure 2:

Infective SARS-CoV-2 viral load in children. (**A**) Viral loads from nasopharyngeal, oropharyngeal, and blood were quantified within SARS-CoV-2 (+), MIS-C, and SARS-CoV-2(−) cohorts. Viral load in nasopharyngeal and oropharyngeal specimens from SARS-CoV-2 (+) children were compared with Mann-Whitney U-test, median presented. (**B**) SARS-CoV-2 viral loads were categorized by symptom duration, including asymptomatic period to day 2 of symptoms, days 3-7 of symptoms, and days 7-26 of symptoms; median presented and comparisons by Kruskal-Wallis. Nasopharyngeal viral load was correlated with (**C**) days of symptoms and (**D**) age; Spearman correlation. NP nasopharyngeal, OP oropharyngeal.

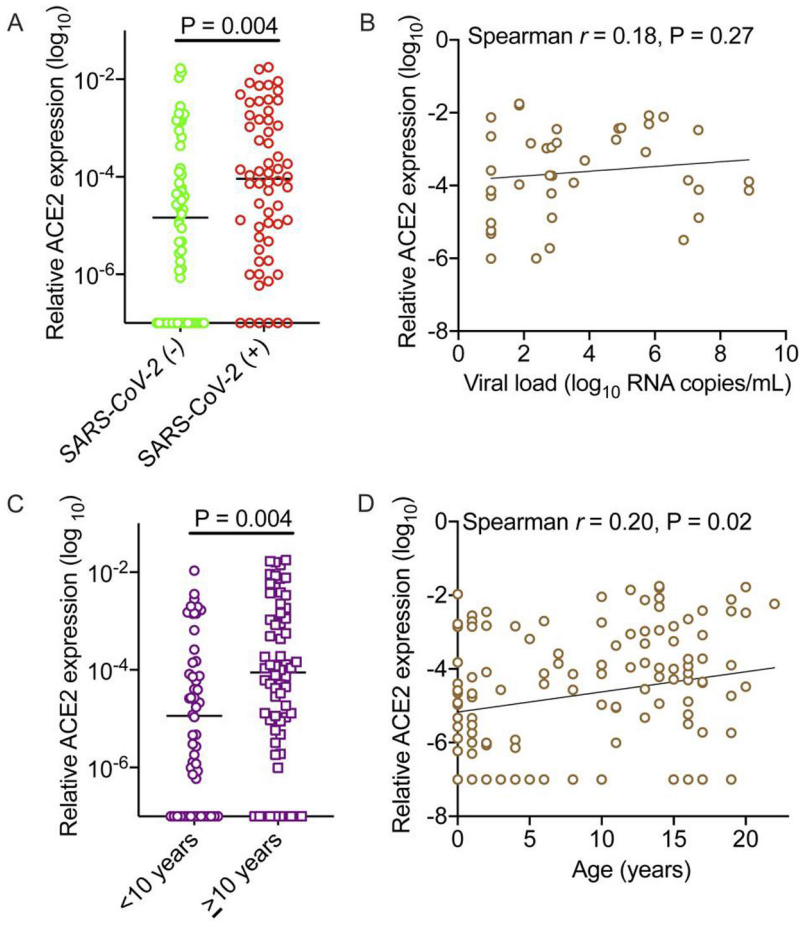

Figure 3:

ACE2 expression in the upper airways of children. (**A**) Relative expression of ACE2 (log_{10}) categorized by SARS-CoV-2 infection, median presented and significance tested by Mann-Whitney U-test. (**B**) Correlation of relative ACE2 expression and viral load (log_{10} RNA copies/ml); Spearman correlation. (**C**) Relative expression of ACE2 (log_{10}) categorized by age <10 years or ≥ 10 years, median presented and significance tested by Mann-Whitney U-test. (**D**) Correlation of ACE2 expression with age; Spearman correlation.

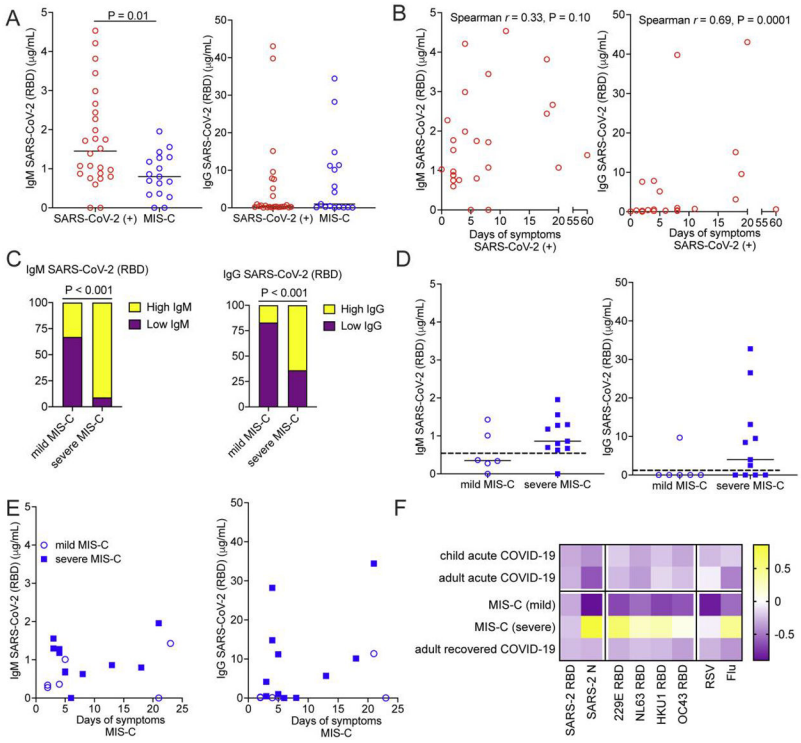

Figure 4:

SARS-CoV-2 antibody response in children infected with SARS-CoV-2. (**A**) Peak IgM and IgG to the RBD component of SARS-CoV-2 were quantified for children acutely infected with SARS-CoV-2 and children presenting with MIS-C. Comparison by Mann-Whitney U-tests; median presented. (**B**) IgM and IgG responses in acute SARS-CoV-2 infection were correlated with days of symptoms; Spearman correlation. (**C**) Percent of children mild vs severe MIS-C with elevated IgM or IgG (above a threshold of 0.5μg/ml) were compared by Fisher exact test. (**D**) Peak IgM and IgG levels were compared between mild and severe MIS-C, Mann-Whitney U-tests; median presented. Dotted line represents 0.5μg/ml threshold for defining high or low antibody response, (**E**) IgM and IgG responses in acute SARS-CoV-2 infection were correlated with days of symptoms; Spearman correlation. (**F**) Heat map depicts relative IgG responses to SARS-CoV-2 RBD and SARS-CoV-2 N capsid protein, other coronaviruses (strains 229E, NL63, HKU1, and OC43), and RSV and influenza (flu). In addition to showing antibody response for children with acute SARS-CoV-2 infection and mild and severe MIS-C, antibody levels from adults with acute SARS-CoV-2 and adults recovered from SARS-CoV-2 infection are displayed.

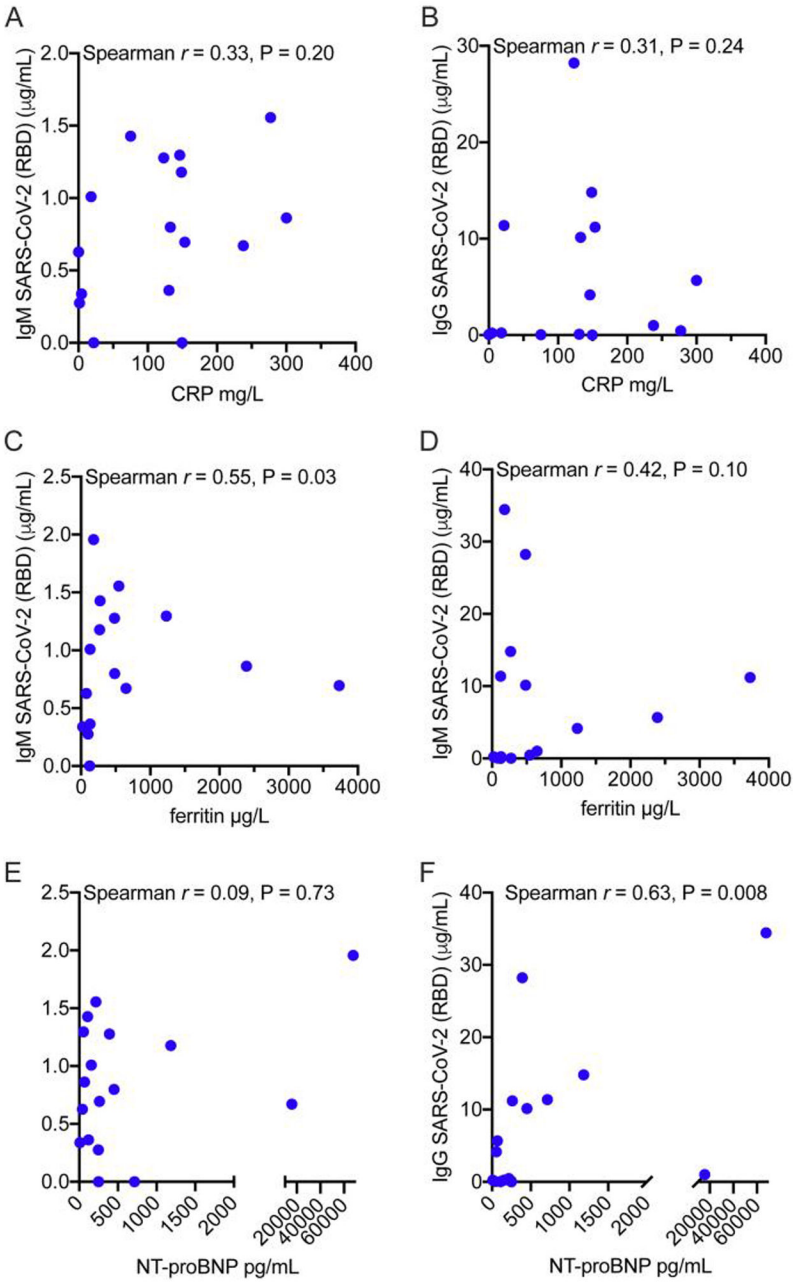

Figure 5; online:

Violin plot of IgM antibodies to SARS-CoV-2 RBD and SARS-CoV-2 N capsid protein, other coronaviruses (strains 229E, NL63, HKU1 and OC43), and RSV and influenza

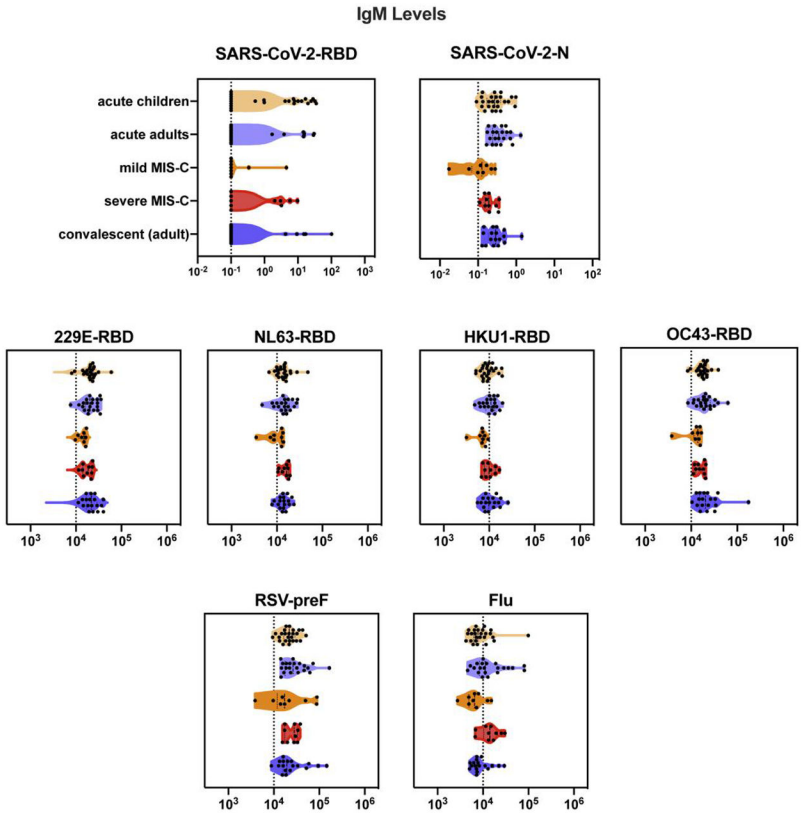

Figure 6; online:

Violin plot of IgG antibodies to SARS-CoV-2 RBD and SARS-CoV-2 N capsid protein, other coronaviruses (strains 229E, NL63, HKU1 and OC4), and RSV and influenza

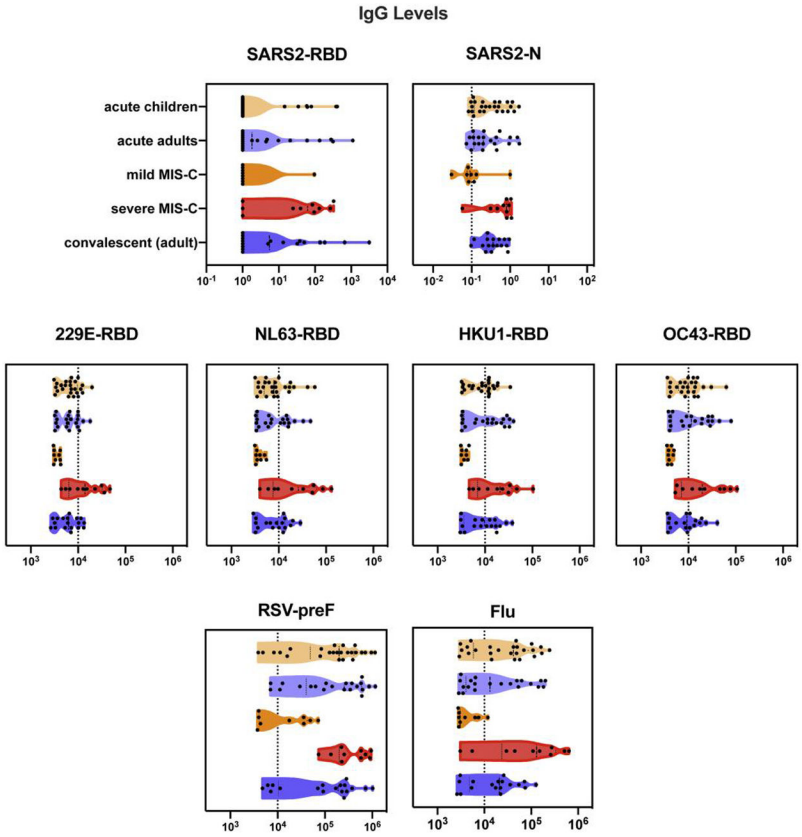

Figure 7; online:

Correlation of inflammatory markers and SARS-CoV-2 antibody responses in MIS-C. IgM and IgG SARS-CoV-2 (RBD component) were correlated with CRP (**A** and **B**, respectively), ferritin (**C** and **D**, respectively), and NT-proBNP (**E** and **F**, respectively); Spearman correlation.

Table 1; online:

Description of adult samples included for comparative purposes in virology and antibody assays.

Patient samples for Virology Assays	
N=162	**Adult patients, hospitalized for COVID-19**
Age, years- mean (SD)	58 (16)
Male sex- no. (%)	107 (66.05)
BMI- mean (SD)	29.3 (6.6)
Past Medical History- no. (%)	
Hypertension	97 (60)
Active Cancer	3 (2)
Chronic Lung Disease	32 (20)
Diabetes	75 (46)
Intubated- no (%)	100 (62)
Death	22 (14)
Patient samples for Antibody Assays	
N=39	**Adults in antibody study**
Age years, mean (SD)	39 (16)
Urgent Care, total no. (%)	21 (54)
SARS-CoV-2 (+), no. (%)	12 (57)
Recovered, no. (%)	18 (46)

Table 2; online:

Patient characteristics of children not infected with SARS-CoV-2, children with SARS-CoV-2 infection, and children diagnosed with MIS-C. Age, sex, socioeconomic status, race and ethnicity, past medical history, vaccination status, COVID-19 household exposures, and daycare/school levels are presented.

Patient Characteristics Total N=192	SARS-CoV-2 (−) n=125	SARS-CoV-2 (+) n=49	MIS-C n=18
Age- avg (SD)	9.6 (7.1)	12.7 (6.3)	7.7 (7.0)
Age group- no. (%)			
<1 year	11 (8.8)	2 (4.3)	2 (11.1)
1-4 years	32 (25.6)	5 (10.6)	7 (38.9)
5-10 years	29 (23.2)	11 (23.4)	4 (22.2)
11-16 years	26 (20.8)	16 (34.0)	2 (11.1)
17-22 years	27 (21.6)	13 (27.7)	3 (16.7)
Male sex- no. (%)	67 (53.6)	23 (46.9)	14 (77.8)
Race- no. (%)			
American Indian/ Alaska Native	0 (0)	0 (0)	0 (0)
Asian	7 (5.6)	1 (2.0)	1 (5.6)
Black or African American	5 (4.0)	4 (8.2)	2 (11.1)
Native Hawaiian/ Pacific Islander	0 (0)	0 (0)	0 (0)
White	43 (34.4)	7 (14.3)	9 (50.0)
Unknown	26 (20.8)	10 (20.4)	2 (11.1)
Ethnicity- no. (%)			
Latino/ Hispanic	63 (50.4)	29 (59.2)	6 (33.3)
Non-Latino/ Non-Hispanic	43 (34.4)	11 (22.4)	10 (55.6)
Past Medical History- no. (%)			
History of Cardiac or Metabolic Disease			
Congenital heart disease	4 (3.2)	0 (0)	0 (0)
Hypertension	3 (2.4)	0 (0)	0 (0)
Diabetes Type 1	1 (0.8)	0 (0)	0 (0)
Diabetes Type 2	0 (0)	0 (0)	0 (0)
Dyslipidemia	0 (0)	2 (4.1)	0 (0)
Obesity	12 (9.6)	13 (26.5)	2 (11.1)
History of Pulmonary Disease			
Asthma	26 (20.8)	6 (12.2)	2 (11.1)
Pneumonia	5 (5.6)	3 (6.1)	0 (0)

Patient Characteristics Total N=192	SARS-CoV-2 (−) n=125	SARS-CoV-2 (+) n=49	MIS-C n=18
History of preterm delivery	11 (8.8)	2 (4.1)	1 (5.6)
Cystic fibrosis	0 (0)	0 (0)	0 (0)
History of Immune/Autoimmune Disease			
Rheumatologic disease	1 (0.8)	0 (0)	0 (0)
Inflammatory bowel disease	0 (0)	1 (2.0)	1 (5.6)
Immunodeficiency	0 (0)	0 (0)	0 (0)
History of Neuro/Neurodevelopmental Disorders			
Seizure	5 (4.0)	7 (14.3)	0 (0)
ADHD	12 (9.6)	5 (10.2)	1 (5.6)
Autism	2 (1.6)	1 (2.0)	1 (5.6)
Cerebral palsy	0 (0)	2 (4.1)	0 (0)
Down Syndrome	1 (0.8)	0 (0)	0 (0)
Vaccinations up to date- no. (%)	101 (80.8)	41 (83.7)	14 (77.8)
Household exposures- no. (%)			
Mother	21 (16.8)	20 (40.8)	4 (22.2)
Father	11 (8.8)	13 (26.5)	2 (11.1)
Sibling	8 (6.4)	9 (18.4)	1 (5.6)
Other	19 (15.2)	9 (18.4)	5 (27.8)
No household exposure	70 (56.0)	9 (18.4)	10 (55.6)
Daycare/School- no. (%)			
Nanny/home daycare	27 (21.6)	6 (12.2)	7 (38.9)
Group daycare	7 (5.6)	1 (2.0)	1 (5.6)
Preschool/kindergarten	7 (5.6)	0 (0)	0 (0)
Grade school	48 (38.4)	26 (53.1)	6 (33.3)
College	4(3.2)	2 (4.1)	0 (0)
Unknown	32 (25.6)	14 (28.6)	4 (22.2)

Table 3; online:

Presenting symptoms of enrolled patients. Comparisons between symptoms reported in acute SARS-CoV-2 infection and non-SARS-CoV-2 illnesses, and SARS-CoV-2 (+) and MIS-C are compared by Fisher exact test.

	Comparison of SARS-CoV-2 (−) and (+)			*Comparison of acute SARS-CoV-2 (+) and MIS-C*	
Symptom report- no. (%)	**SARS-CoV-2 (−)**	**SARS-CoV-2 (+)**	**p-value**	**MIS-C**	**p-value**
Congestion	27 (21.6)	17 (34.7)	*0.08*	0 (0)	*0.002*
Rhinorrhea	27 (21.6)	14 (28.6)	*0.33*	0 (0)	*<0.001*
Anosmia/Hyposmia	3 (2.4)	10 (20.4)	*<0.001*	1 (5.6)	*0.005*
Headache	30 (24.0)	13 (26.5)	*0.75*	2 (11.1)	*0.006*
Myalgia/Arthralgia	26 (20.8)	14 (28.6)	*0.25*	3 (16.7)	*0.06*
Sore throat	26 (20.8)	17 (34.7)	*0.04*	2 (11.1)	*<0.001*
Cough	49 (39.2)	23 (46.9)	*0.32*	4 (22.2)	*0.003*
Fever	59 (47.2)	25 (51.0)	*0.67*	18 (100.0)	*<0.001*
Rash	11 (8.8)	1 (2.0)	*0.06*	5 (27.8)	*<0.001*
Nausea/vomiting	17 (13.6)	3 (6.1)	*0.10*	5 (27.8)	*<0.001*
Diarrhea	12 (9.6)	3 (6.1)	*0.44*	3 (16.7)	*0.02*
Anorexia	6 (4.8)	3 (6.1)	*>0.99*	1 (5.6)	*>0.99*
Chills	2 (1.6)	4 (8.2)	*0.10*	2 (11.1)	*0.63*
Dyspnea	17 (13.6)	8 (16.3)	*0.84*	3 (16.7)	*>0.99*
Fatigue	4 (3.2)	2 (4.1)	*>0.99*	1 (5.6)	*0.75*
Dysgeusia	1 (0.8)	3 (6.1)	*0.12*	0 (0)	*0.03*
Altered mental status	1 (0.8)	0 (0)	*>0.99*	0 (0)	*>0.99*
Lymphadenopathy	0 (0)	0 (0)	*N/a*	0 (0)	*N/a*