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Impact of SARS-CoV-2 and COVID-19 pandemic in pediatrics

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Impact of SARS-CoV-2 and COVID-19 pandemic in pediatrics

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Factors Associated With Hospitalization or Intensive Care Admission in Children With COVID-19 in Latin America

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Background: Limited data is available from low-middle and upper-middle income countries of the factors associated with hospitalization or admission to pediatric intensive care unit (PICU) for children with COVID-19.

Objective: To describe the factors associated with hospitalization or PICU admission of children with COVID-19 in Latin America.

Method: Multicenter, analytical, retrospective study of children reported from 10 different Latin American countries to the Latin-American Society of Pediatric Infectious Diseases (SLIPE-COVID) research network from June 1, 2020, and February 28, 2021. Outpatient or hospitalized children <18 years of age with COVID-19 confirmed by polymerase chain reaction or antigen detection from the nasopharynx were included. Children with multisystem inflammatory syndrome in children (MIS-C) were excluded. Associations were assessed using univariate and multivariable logistic regression models.

Results: A total of 1063 children with COVID-19 were included; 500 (47%) hospitalized, with 419 (84%) to the pediatric wards and 81 (16%) to the ICU. In multivariable analyses, age <1 year (Odds Ratio [OR] 1.78; 95% CI 1.08–2.94), native race (OR 5.40; 95% CI 2.13–13.69) and having a co-morbid condition (OR 5.3; 95% CI 3.10–9.15), were associated with hospitalization. Children with metabolic or endocrine disorders (OR 4.22; 95% CI 1.76–10.11), immune deficiency (1.91; 95% CI 1.05–3.49), preterm birth (OR 2.52; 95% CI 1.41–4.49), anemia at presentation (OR 2.34; 95% CI 1.28–4.27), radiological peribronchial wall thickening (OR 2.59; 95% CI 1.15–5.84) and hypoxia, altered mental status, seizures, or shock were more likely to require PICU admission. The presence of pharyngitis (OR 0.34; 95% CI 0.25–0.48); myalgia (OR 0.47; 95% CI 0.28–0.79) or diarrhea (OR 0.38; 95% CI 0.21–0.67) were inversely associated with hospital admission.

Conclusions: In this data analysis reported to the SLIPE research network in Latin America, infants, social inequalities, comorbidities, anemia, bronchial wall thickening and specific clinical findings on presentation were associated with higher rates of hospitalization or PICU admission. This evidence provides data for prioritization prevention and treatment strategies for children suffering from COVID-19.

Keywords: COVID-19, SARS-CoV-2, children, critical care, hospitalization

INTRODUCTION

The emergence and rapid global spread of SARS-CoV-2 in early 2020 posed a major threat to populations from low and middle income countries (LMIC), where data on clinical presentations and risk factors for serious COVID-19 infections in children were scarce (1, 2). There are now ∼64 million cases of COVID-19 in Latin America (3), with 8–16% of these cases reported among those between 0 and 19 years of age in different countries of the region (4–8). Although COVID-19 presents as mild or asymptomatic in most children, severe disease and hospitalization can occur in ∼1% of children and is likely to increase as children represent most of the unvaccinated in these countries (9).

Early identification of factors associated with severe disease in children allows clinicians and public health officials to triage those in need of advanced level of care and is critical as vaccination campaigns are rolled out, allowing the prioritization of those who will benefit the most from early protection. Although several observational studies have provided information on risk factors for hospitalization or critical care admission in children in high-income countries (10–16), detailed demographic, laboratory, radiological and clinical data obtained from a large sample in LMIC countries are scarce.

Pediatric societies around the world have established data registries of pediatric COVID-19 cases to understand the epidemiology, clinical presentation and morbidity and mortality rates in children (9, 17). The Sociedad Latinoamericana de Infectologia Pediatrica (SLIPE) started a multinational collaboration to create a data registry on children being affected with COVID-19 in Latin American countries. The purpose of the present study was to describe the factors associated with hospitalization or pediatric intensive care unit (PICU) admission in children with COVID-19 in Latin America.

MATERIALS AND METHODS

Independent pediatricians collected and reported cases of COVID-19 using an online registry created by the SLIPE-COVID network that utilized a standardized data collection form developed in Research Electronic Data Capture (REDCap) hosted by the University of Colorado, Aurora, USA. Investigators from 17 sites in 14 cities from 10 different Latin American countries prospectively identified patients between June 1, 2020, and February 28, 2021 and retrospectively inputted their data. All study sites were level 3 referral hospitals. The registry and study protocol were reviewed and approved by the local institutional review board of each participating center. Since data was collected for routine clinical practice, research was deemed of minimal risk and the requirement for informed consent was waived.

Children <18 years of age who presented to outpatient clinics or hospital emergency departments from participating sites with COVID-19-related symptoms and polymerase chain reaction (PCR) or antigen-confirmed SARS-CoV-2 infection were included. Children with MIS-C were excluded from this analysis.

Case ascertainment varied across sites. Seven cities used an active case surveillance system that allowed to identify children with confirmed COVID-19 from a list of all ambulatory or hospitalized encounters provided by the hospital's epidemiology or microbiology department, while a passive case ascertainment method was used in the remaining seven cities where children

were identified after a pediatric infectious disease service consultation was obtained.

Patient characteristics including demographics, clinical, laboratory and radiological data were collected from medical records as documented by the site clinician who evaluated the patient. For children with one or more COVID-19-related consultations during the study period, only data from the first encounter was collected and for children with multiple laboratory reports during their care, only first values were recorded. Nutritional status was classified based on body mass index (BMI) standard deviations (SD) according to the World Health Organization (WHO) into underweight (below −2 SD), normal nutritional status (between −2 and +1 SD), overweight (between +1 and +2 SD) and obesity (above +2 SD) (18). Radiologic findings were categorized according to the density and extent of parenchymal changes. Anemia was defined as hemoglobin below 11 grams/deciliter (19) and thrombocytopenia as platelets <150,000 × 10^3 cells/μL (20). C-reactive protein and neutrophil to lymphocyte ratio were dichotomized as previous reports (21–23). For symptoms that were not described in medical records, investigators recorded "non-applicable" when children were too young or too ill to describe their symptoms.

Outcome endpoints for this analysis were divided into (a) outpatient care, (b) hospitalization in a general pediatric ward, or (c) intensive care unit (ICU) admission. Final disposition was at the discretion of primary care team. Data were compared in children requiring admission in the general ward vs. ICU, in those requiring hospitalization (general ward or ICU) vs. outpatient care and in those who did or did not require supplementary oxygen. In the latter two groups laboratory or imaging data were not compared, as children receiving outpatient care seldom had blood drawn or chest X-rays obtained.

Data Analysis

Summary statistics were used for description of variables. Medians and interquartile ranges (IQR) were used for continuous variables; categorical variables were reported as absolute numbers and proportions. Chi^2 tests and Fisher's exact tests were used for categorical variables as appropriate, and the Mann-Whitney U test was used for comparing median values of non-normally distributed variables. Associations of baseline characteristics and clinical findings with hospitalization, PICU and/or supplementary oxygen requirement were assessed using univariate and multivariable logistic regression models. Covariates that were significant at the 0.20 level in univariate analysis were included in multivariable models. A backward elimination algorithm was performed to select covariates that were independently associated with hospitalization, PICU and/or supplementary oxygen use, setting a p value of <0.05 as significant. Backward variable selection was performed using Rubin's Rules (24). Two multivariate models were created to explore associations with the outcomes of interest. One explored sociodemographic characteristics and comorbidities, and another explored signs/symptoms, laboratory values and radiologic findings. A sensitivity analysis including only children from the seven cities that used an active surveillance system was conducted. In addition, all co-morbidities were integrated in a model that explored the effect of any co-morbidity in the risk of hospital admission or PICU.

For missing data, a multiple imputation chained equation with 90 imputed datasets was used. This value was set using the rule of thumb (25). All variables, including the binary outcomes, were included in the imputation model and further sensitivity analysis was conducted using complete-case data. To control for variability in case ascertainment and non-measured social or cultural confounders, clustered standard errors were estimated to adjust for the correlation between children from the same city in each model. Hospitals from the same city had the same case ascertainment method. Variables that could not be assessed in all children due to age (i.e. anosmia or dysgeusia) were not imputed nor included in multivariable analysis. All statistical analyses were carried out in Stata version 16.0 (StataCorp, College, Station, TX) and p values of <0.05 (two-sided) were considered statistically significant. Imputation was not attempted for variables that had more than 50% of their data missing in the complete dataset except for the C-reactive protein values; this variable was imputed given its relevance in predicting severe outcomes in other studies (23, 26).

RESULTS

Study Participants

A total of 1063 children were included (median age, 3 years [IQR 1–9]; age range 22 days to 17.8 years, 46.4% female, 63% with normal weight and 29% with comorbidity). Of all, 563 (53%) received ambulatory care, while 500 (47%) were hospitalized (419 [84%] in the general ward and 81 [16%] in the ICU) (**Table 1**). Most children were included from 2 hospitals in Bogotá, Colombia (n = 387); 1 hospital in Quilmes, Argentina (n = 264); 2 hospitals in Cali, Colombia (n = 160) and 2 hospitals in Panamá (n = 109). The remaining cases (n = 143) were included from Asunción, Paraguay; David, Panamá; Guatemala City, Guatemala; La Paz, Bolivia; Lara, Venezuela; Monterrey, México; Rosario, Argentina; San Lorenzo, Paraguay; San Salvador, El Salvador and Uberlandia, Brazil (**Figure 1**).

Most children had fever (70%) and cough (55%). Fewer than one third had signs or symptoms of upper respiratory tract infection (**Table 1**). Ninety-seven children (9%) had diarrhea without respiratory symptoms while 104 children (9.8%) did not have either fever or respiratory symptoms at admission. The frequency of these clinical presentations varied by age groups (**Supplementary Table 1**). Frequency of missing data is presented in **Supplementary Table 2**.

Eighteen children died (median age 6.2 years [IQR 1.8–12.2], 11 [61%] males), 11 (61%) had comorbidities (mainly immune deficiency [n = 6, 33%] or neurologic [n = 3, 17%]), and 22% came from a rural area. The majority presented with fever (n = 15, 83%), hypoxia (n = 14, 78%), dyspnea (n = 13, 72%) and cough (n = 11, 61%). Characteristics of hospital admission among 500 hospitalized children are described in **Supplementary Table 3**.

TABLE 1 | Demographics and clinical characteristics of pediatric patients with COVID-19.

Characteristic	All patients	Outpatient care	Hospitalized	
			General ward	PICU
	(*n* = 1,063)	(*n* = 563)	(*n* = 419)	(*n* = 81)
Age, median (IQR), yr	3 (1–9)	4 (1–10)	3 (1–9)	3 (1–9)
Age groups, yr No. (%)				
<1	241 (22.7)	109 (19.4)	113 (27.0)	19 (23.5)
1–5	386 (36.3)	214 (38.0)	141 (33.6)	31 (38.3)
6–9	176 (16.6)	95 (16.9)	67 (16.0)	14 (17.3)
≥10	260 (24.5)	145 (25.7)	98 (23.4)	17 (21.0)
Sex, No. (%)				
Male	570 (53.6)	291 (51.7)	237 (56.6)	42 (51.9)
Female	493 (46.4)	272 (48.3)	182 (43.4)	39 (48.1)
Race or ethnic group[a], No. (%)				
No.*	987	517	396	74
Caucasian	459 (46.5)	285 (55.1)	149 (37.6)	25 (33.8)
Native	16 (1.6)	3 (0.6)	11 (2.8)	2 (2.7)
Black or African American	9 (0.9)	6 (1.2)	3 (0.8)	0 (0.0)
Mixed race	503 (51.0)	223 (43.1)	233 (58.8)	47 (63.5)
Population, No. (%)				
No.*	880	415	385	80
Urban	807 (91.7)	393 (94.7)	347 (90.1)	67 (83.8)
Rural	73 (8.3)	22 (5.3)	38 (9.9)	13 (16.2)
Level of Education of caregiver, No. (%)				
No.*	248	115	110	23
No education	87 (35.1)	25 (21.7)	53 (48.2)	9 (39.1)
Primary education	40 (16.1)	17 (14.8)	18 (16.4)	5 (21.7)
High school	79 (31.8)	40 (34.8)	32 (29.1)	7 (30.4)
University degree	42 (16.9)	33 (28.7)	7 (6.4)	2 (8.7)
Full immunization coverage, No. (%)				
No.*	859	477	310	72
Yes	770 (89.6)	435 (91.2)	274 (88.4)	61 (84.7)
Nutritional Status[b], No. (%)				
No.*	562	259	242	61
Underweight	65 (11.6)	17 (6.6)	37 (15.3)	11 (18.0)
Normal Weight	356 (63.3)	173 (66.8)	149 (61.6)	34 (55.7)
Overweight	83 (14.8)	47 (18.1)	31 (12.8)	5 (8.2)
Obese	58 (10.3)	22 (8.4)	25 (10.3)	11 (18)
Co-morbidities- No. (%)				
Chronic lung disease	123 (11.6)	54 (9.6)	58 (13.8)	11 (13.6)
Congenital heart disease	18 (1.7)	6 (1.1)	9 (2.1)	3 (3.7)
Cirrhosis/Biliary atresia	1 (0.1)	1 (0.2)	0 (0.0)	0 (0.0)
Chronic gastrointestinal disease	9 (0.8)	2 (0.4)	6 (1.4)	1 (1.2)
Renal insufficiency	8 (0.7)	0 (0.0)	8 (1.9)	0 (0.0)
Neurologic disease	59 (5.5)	7 (1.2)	41 (9.8)	11 (13.6)
Metabolic or endocrine disorder	8 (0.7)	1 (0.2)	4 (0.9)	3 (3.7)
Immune deficiency	88 (8.3)	13 (2.3)	57 (13.6)	18 (22.2)
Preterm birth	33 (3.1)	9 (1.6)	17 (4.1)	7 (8.6)
Any co-morbidity- No. (%)	305 (28.7)	84 (14.9)	175 (41.8)	46 (56.8)
Source of infection- No. (%)				
Hospital	27 (2.5)	1 (0.2)	19 (4.5)	7 (8.6)
Community	79 (7.4)	26 (4.6)	42 (10.0)	11 (13.6)

(Continued)

TABLE 1 | Continued

Characteristic	All patients	Outpatient care	Hospitalized	
			General ward	PICU
	(*n* = 1,063)	(*n* = 563)	(*n* = 419)	(*n* = 81)
Traveling	5 (0.5)	2 (0.4)	3 (0.7)	0 (0.0)
Home	376 (35.4)	248 (44.0)	113 (27.0)	248 (44.0)
Other	6 (0.6)	4 (0.7)	1 (0.2)	1 (1.2)
Unknown	585 (55.0)	290 (51.5)	247 (58.9)	48 (59.3)
Symptoms or Signs described or present on admission- No. (%)				
Anosmia	25 (3.4)	19 (4.7)	3 (1.1)	3 (5.1)
No.*	745, NA = 318	403, NA = 160	283, NA = 136	59, NA = 22
Dysgeusia	27 (3.6)	19 (4.7)	6 (2.1)	2 (3.3)
No.*	756, NA = 307	407, NA = 156	289, NA = 130	60, NA = 21
Skin rash	34 (3.2)	11 (2.0)	21 (5.0)	2 (2.5)
No.*	1,059	562	416	81
Conjunctivitis	15 (1.4)	8 (1.4)	6 (1.4)	1 (1.2)
No.*	1,059	562	416	81
Abdominal pain	127 (12.6)	62 (11.4)	54 (13.7)	11 (14.9)
No.*	1,011, NA = 51	543, NA = 20	394, NA = 24	74, N = 7
Fever	745 (70.1)	424 (75.3)	263 (62.9)	58 (71.6)
No.*	1,062	563	418	81
Cough	580 (54.7)	324 (57.5)	209 (50.1)	47 (58.0)
No.*	1,061	563	417	81
Pharyngitis	248 (23.4)	177 (31.4)	59 (14.2)	12 (14.8)
No.*	1,059	563	415	81
Rhinitis	314 (29.6)	181 (32.1)	111 (26.7)	22 (27.2)
No.*	1,060	563	416	81
Headache	162 (15.3)	114 (20.2)	39 (9.4)	9 (11.1)
No.*	1,058	563	414	81
Myalgia	90 (8.5)	60 (10.7)	22 (5.3)	8 (9.9)
No.*	1,055	561	413	81
Malaise	341 (32.2)	182 (32.4)	123 (29.6)	36 (44.4)
No.*	1,058	562	415	81
Diarrhea	219 (20.7)	139 (24.7)	61 (14.7)	19 (23.5)
No.*	1,059	563	415	81
Vomit	199 (18.8)	107 (19.0)	77 (18.6)	15 (18.5)
No.*	1,059	563	415	81
Dyspnea	231 (21.8)	41 (7.3)	137 (32.8)	53 (65.4)
No.*	1,060	561	418	81
Hypoxia	175 (16.5)	8 (1.4)	115 (27.5)	52 (64.2)
No.*	1,060	561	418	81
Hemoptisis	5 (0.5)	1 (0.2)	3 (0.7)	1 (1.2)
No.*	1,058	561	416	81
Altered mental status	43 (4.1)	4 (0.7)	13 (3.1)	26 (32.1)
No.*	1,058	561	416	81
Seizures	46 (4.3)	8 (1.4)	21 (5.0)	17 (21.2)
No.*	1,058	561	417	80
Dehydration	67 (6.3)	7 (1.2)	41 (9.9)	19 (23.7)
No.*	1,055	560	415	80
Shock	28 (2.6)	1 (0.2)	5 (1.2)	22 (27.2)
No.*	1,057	559	417	81
Onset of symptoms to, median (IQR)	2 (1–3)	1 (0–3)	1 (0–3)	1 (1–3)

IQR, Interquartile range; PICU, pediatric intensive care unit.

[a] *Race/ethnic group was collected by study personnel based on auto reporting by the study participants. "Mixed race" refers to an individual of mixed European/Native heritage.*

[b] *Nutritional status was classified based on standard deviations for body mass index according to the World Health Organization into thinness (below −2 standard deviations [SD]), normal nutritional status (between −2 and +1 SD), overweight (between +1 and +2 SD) and obesity (above +2 SD). Body mass index was calculated as weight in kilograms divided by height in meters squared. *Number of patients with available data.*

FIGURE 1 | SLIPE-COVID research network-city locations and sites.

Risk Factors for Hospital Admission

Five hundred hospitalized children were compared with 563 children who received outpatient care. In the multivariable model using multiple imputation, younger age (<1 year), native race, and the presence of certain underlying medical conditions were associated with an increased risk for hospital admission (**Table 2** and **Figure 2**). In a model that considered all underlying medical conditions together, the odds for hospital admission were 5.3 times higher (95% CI 3.10 to 9.15) in children with any underlying medical conditions. Both sensitivity analysis (using the complete dataset that included missing data and only children who were enrolled from the seven cities that used an active surveillance system) showed similar results (**Supplementary Table 4**).

Children presenting with skin rash, abdominal pain, dehydration, hypoxia, or seizures were at increased risk for hospital admission, while pharyngitis, myalgia and diarrhea reduced the odds of hospital admission (**Table 3**). Similar results were obtained from both sensitivity analysis (**Supplementary Table 5**).

Risk Factors for ICU Admission

The 81 children who required ICU admission were compared with the remaining 419 children who were hospitalized in the general pediatric ward. In the multiple imputation model, residing in a rural area was the only sociodemographic factor associated with increased odds of ICU care, while metabolic or endocrine disorder, immune deficiency and preterm birth were the underlying medical conditions significantly associated with ICU admission (**Table 4**). Sensitivity analysis showed similar results (**Supplementary Table 6**).

Children presenting with diarrhea, hypoxia, altered mental status, seizures or shock were at higher risk for ICU admission. Anemia and bronchial wall thickening in chest radiograph were also associated with increased risk (**Table 5**). Sensitivity analysis showed similar results (**Supplementary Table 7**).

Risk Factors Associated With Supplementary Oxygen Use

Risk factors associated with supplementary oxygen requirement were similar to those associated with hospital admission. In addition, history of chronic lung disease increased the risk for supplementary oxygen requirement (OR 4.45, 95% CI 2.35–8.41) (**Supplementary Table 8**).

DISCUSSION

In this multi-country case registry of children with COVID-19 (excluding MIS-C), we identified several factors independently associated with ICU admission, hospitalization and need for supplementary oxygen. Similar to other reports, age under 1-year, comorbidities such as metabolic/endocrine disorders, preterm birth or immune deficiency, certain presenting signs/symptoms and social determinants of health were associated with increased COVID-19-related morbidity (10, 15, 27–30). In addition, low hemoglobin concentrations and bronchial wall thickening on chest radiographs were independently associated with the need for PICU admission.

Social determinants of health have long been recognized to be important predictors as to how epidemics are experienced in terms of infection rates and morbidity (31), particularly in more socially unequal regions, such as Latin America. A multicenter cohort study reported that rates of pediatric PICU admission and deaths due to COVID-19 were higher in Latin American than European children (32). Disparities in health determinants like economic instability, insurance status and housing conditions of patients and their families have consistently placed social, racial, and ethnic minorities at greater risk for severe illness by COVID-19 (31). This is especially true for children and their social determinants influencing life opportunities, disease characteristics and health outcomes. We described greater risks in children with demographic characteristics indicative of lower socio-economic status in Latin America such as native ethnic group or living in rural areas. Social inequalities and low socioeconomic status have also been described as risk factors for death (28), so it is possible that unmeasured socioeconomic or cultural disparities that increase the risk of a more severe or late presentation in children living in rural areas may have been present in this study. For example, although level of education of the caregiver was not imputed or included in multivariable analysis due to the high frequency of missing data, there was a higher proportion of uneducated caregivers among those children who required hospitalization.

Obesity and diabetes mellitus are comorbidities that have been identified as risk factors for disease severity in other studies

TABLE 2 | Demographic and underlying medical conditions associated with hospitalization (general ward and intensive care), among pediatric patients with COVID-19 using multiple imputation chained equations for missing data.

Characteristic	Outpatient care (*n* = 563)	Hospitalization (*n* = 500)	Univariate analysis OR (95% CI)	Multivariable analysis OR (95% CI)
Age groups, yr. No. (%)				
<1	109 (19.4)	132 (26.4)	1.42 (0.86–2.34)	1.78 (1.08–2.94)
1–5	214 (38.0)	172 (344.4)	0.94 (0.75–1.18)	1.14 (0.87–1.50)
6–9	95 (16.9)	81 (16.2)	Ref.	Ref.
≥10	145 (25.7)	115 (23.0)	0.93 (0.66–1.31)	0.89 (0.60–1.34)
Sex, No. (%)				
Male	291 (51.7)	279 (55.8)	Ref.	-
Female	272 (48.3)	221 (44.2)	0.85 (0.66–1.06)	-
Race or ethnic group[a], No. (%)				
Caucasian	285 (55.1)	174 (37.0)	Ref.	Ref.
Native	3 (0.6)	13 (2.8)	6.03 (1.99–18.22)	5.40 (2.13–13.69)
Black or African American	6 (1.2)	3 (0.6)	0.90 (0.11–7.07)	0.96 (0.16–5.66)
Mixed race	223 (43.1)	280 (59.6)	2.02 (0.55–7.14)	1.86 (0.59–5.88)
Population, No. (%)				
Urban	393 (94.7)	414 (89.0)	Ref.	-
Rural	22 (5.3)	51 (11.0)	1.95 (1.14–3.35)	-
Full immunization coverage, No. (%)				
Yes	435 (91.2)	335 (87.7)	0.63 (0.30–1.35)	-
Nutritional Status[b], No. (%)				
Underweight	17 (6.6)	48 (15.8)	1.57 (0.85–2.90)	-
Normal Weight	173 (66.8)	183 (60.4)	Ref.	-
Overweight	47 (18.1)	36 (11.9)	0.83 (0.54–1.28)	-
Obese	22 (8.5)	36 (11.9)	1.13 (0.67–1.93)	-
Co-morbidities- No. (%)				
Chronic lung disease	54 (9.6)	69 (13.8)	1.51 (0.75–3.04)	-
Congenital heart disease	6 (1.1)	12 (2.40)	2.28 (0.55–9.51)	-
Chronic gastrointestinal disease	2 (0.4)	7 (1.40)	3.98 (1.40–11.31)	3.31 (1.18–9.29)
Neurologic disease	7 (1.2)	52 (10.4)	9.22 (3.80–22.34)	10.77 (3.97–29.81)
Metabolic or endocrine disorder	1 (0.2)	7 (1.40)	7.98 (0.99–64.09)	10.53 (1.77–62.79)
Immune deficiency	13 (2.3)	75 (15.0)	7.47 (2.78–20.07)	9.05 (3.24–25.27)
Preterm birth	9 (1.6)	24 (4.8)	3.10 (1.58–6.10)	2.19 (1.14–4.22)

OR, odds ratio; IQR, interquartile range.

[a]Race/ethnic group was collected by study personnel based on auto reporting by the study participants. "Mixed race" refers to an individual of mixed European/Native heritage.

[b]Nutritional status was classified based on standard deviations for body mass index according to the World Health Organization into thinness (below −2 standard deviations [SD]), normal nutritional status (between −2 and +1 SD), overweight (between +1 and +2 SD) and obesity (above +2 SD). Body mass index was calculated as weight in kilograms divided by height in meters squared.

(12, 15, 33–35). In this study, obesity was not significantly associated with higher risk of hospitalization or ICU admission. However, obesity was more frequent in children requiring ICU admission than in children requiring outpatient care (18 vs. 8.4%, respectively), although no multivariable model comparison was made for these two categories. In addition, obese children were at higher odds of hospitalization (overall and in the ICU), although this was of limited statistical significance. Yet, in studies from high-income countries, obesity has been consistently demonstrated as a risk factor for disease severity in children, especially in adolescents (15, 36). In animal studies, angiotensin-converting enzyme 2 (ACE2) protects against SARS-CoV-2 associated acute respiratory distress syndrome (ARDS) (37). ACE2 expression is decreased in children with diabetes mellitus likely due to glycosylation (38), which may explain their higher risk for hospital or ICU admission in this and other series (33). Although asthma has been suggested as a risk factor for severe illness in children with COVID-19, our study, as well as a registry-based (39) and a cohort study (16) did not confirm this association. Overall, the underlying medical conditions associated with hospital or ICU admission in this Latin American pediatric registry are similar to those described for the same outcomes in US children (39).

As with children with COVID-19 from European and North American countries (11, 40), fever and cough were the predominant clinical features at presentation (70 and 55%,

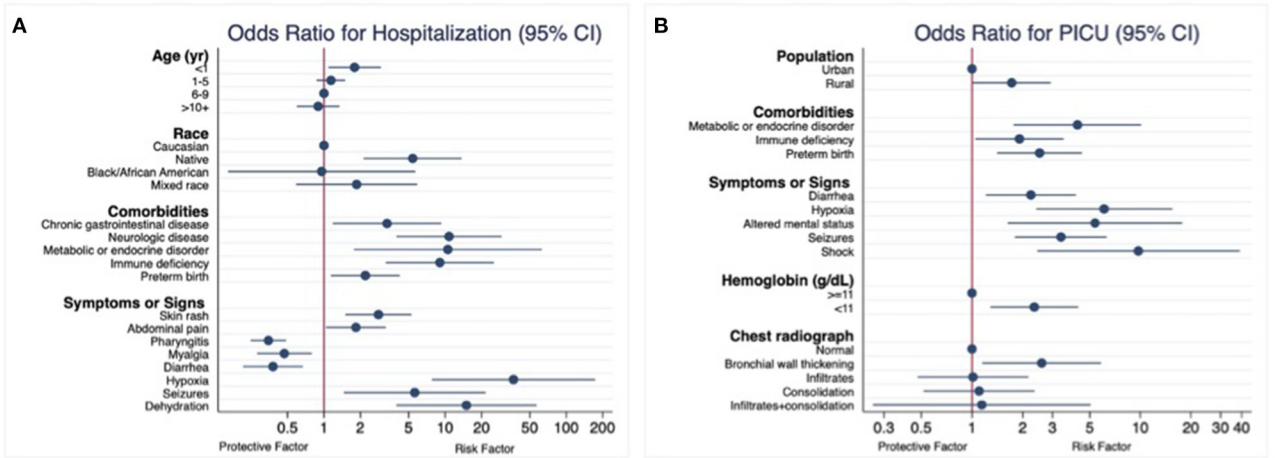

FIGURE 2 | Risk factors among pediatric patients with COVID-19. Multiple imputation chained equations for missing data. **(A)** Hospital admission (general pediatric ward or pediatric intensive care unit). **(B)** Pediatric intensive care admission among hospitalized patients.

TABLE 3 | Signs or symptoms described or present on initial evaluation associated with hospitalization (general ward and intensive care), among pediatric patients with COVID-19 using multiple imputation chained equations for missing data.

Symptoms or Signs[a] - No. (%)	Outpatient care (*n* = 563)	Hospitalization (*n* = 500)	Univariate analysis OR (95% CI)	Multivariable analysis OR (95% CI)
Anosmia	19 (4.7)	6 (1.8)	-	-
Dysgeusia	19 (4.7)	8 (2.3)	-	-
Skin rash	11 (2.0)	23 (4.6)	2.42 (1.40–4.19)	2.81 (1.51–5.27)
Conjunctivitis	8 (1.4)	7 (1.4)	1.00 (0.47–2.12)	-
Abdominal pain	62 (11.4)	65 (13.9)	1.22 (0.90–1.64)	1.83 (1.04–3.24)
Fever	424 (75.3)	321 (64.3)	0.59 (0.29–1.21)	-
Cough	324 (57.5)	256 (51.4)	0.78 (0.46–1.32)	-
Pharyngitis	177 (31.4)	71 (14.3)	0.36 (0.29–0.45)	0.34 (0.25–0.48)
Rhinitis	181 (32.1)	133 (26.8)	0.77 (0.31–1.90)	-
Headache	114 (20.2)	48 (9.7)	0.42 (0.23–0.77)	-
Myalgia	60 (10.7)	30 (6.1)	0.53 (0.27–1.06)	0.47 (0.28–0.79)
Malaise	182 (32.4)	159 (32.1)	0.98 (0.47–2.08)	-
Diarrhea	139 (24.7)	80 (16.1)	0.58 (0.33–1.03)	0.38 (0.21–0.67)
Vomit	107 (19.0)	92 (18.5)	0.97 (0.55–1.69)	-
Dyspnea	41 (7.3)	190 (38.1)	7.79 (4.61–13.18)	-
Hypoxia	8 (1.4)	167 (33.5)	34.64 (7.58–158.36)	36.69 (7.76–173.39)
Altered mental status	4 (0.7)	39 (7.8)	11.72 (3.57–38.53)	-
Seizures	8 (1.4)	38 (7.6)	5.72 (1.74–18.76)	5.61 (1.46–21.58)
Dehydration	7 (1.2)	60 (12.1)	10.90 (3.85–30.87)	14.99 (3.96–58.84)
Shock	0	27 (5.4)	-	-
Onset of symptoms to, median (IQR)	2 (1–3)	1 (0–3)	1.00 (0.96–1.05)	-

[a] *Laboratory or radiologic data not included due to high frequency of missing data.*

respectively) and approximately a third of children presented with gastrointestinal symptoms. Some clinical manifestations are related to disease progression and complications and are predictive of a higher level of care. As reported in adults (41), children presenting with hypoxia, altered mental status, seizures, shock, dyspnea, or dehydration were more likely to require hospital admission or intensive care. Pharyngitis, myalgia, and diarrhea were identified in this series as inversely associated with hospital admission; and in the complete dataset anosmia and dysgeusia were also found to be protective although of limited statistical significance. A UK study also found that children presenting with upper respiratory signs (rhinorrhea)

TABLE 4 | Demographic and underlying medical conditions associated with intensive care, among pediatric patients hospitalized due to COVID-19 using multiple imputation chained equations for missing data.

Characteristics	Place of hospitalization		Multiple Imputation	
	General ward (*n* = 419)	Pediatric intensive care unit (*n* = 81)	Univariable analysis OR (95% CI)	Multivariable analysis OR (95% CI)
Age groups, yr. No. (%)				
<1	113 (27.0)	19 (23.5)	0.80 (0.53–1.22)	-
1–5	141 (33.6)	31 (38.3)	1.05 (0.66–1.66)	-
6–9	67 (16.0)	14 (17.3)	Ref.	-
≥10	98 (23.4)	17 (21.0)	0.83 (0.47–1.46)	-
Sex, No. (%)				
Male	237 (56.6)	42 (51.9)	Ref.	-
Female	182 (43.4)	39 (48.1)	1.21 (0.73–2.01)	-
Race or ethnic group[a], No. (%)				
Caucasian	149 (37.6)	25 (33.8)	Ref.	-
Native	11 (2.8)	2 (2.7)	1.06 (0.26–4.24)	-
Black or African American	3 (0.8)	0 (0.0)	-	-
Mixed race	233 (58.8)	47 (63.5)	1.22 (0.38–3.88)	-
Population, No. (%)				
Urban	347 (90.1)	67 (83.8)	Ref.	Ref.
Rural	38 (9.9)	13 (16.2)	1.76 (1.03–2.99)	1.72 (1.02–2.92)
Full immunization coverage, No. (%)				
Yes	274 (88.4)	61 (84.7)	0.70 (0.38–1.28)	-
Nutritional Status[b], No. (%)				
Underweight	37 (15.3)	11 (18.0)	1.28 (0.77–2.14)	-
Normal Weight	149 (61.6)	34 (55.8)	Ref.	-
Overweight	31 (12.8)	5 (8.2)	0.69 (0.25–1.89)	-
Obese	25 (10.33)	11 (18.0)	1.36 (0.60–3.10)	-
Co-morbidities- No. (%)				
Chronic lung disease	58 (13.8)	11 (13.6)	0.98 (0.24–3.91)	-
Congenital heart disease	9 (2.1)	3 (3.7)	1.75 (0.54–5.69)	-
Chronic gastrointestinal disease	6 (1.4)	1 (1.2)	0.86 (0.07–10.18)	-
Neurologic disease	41 (9.8)	11 (13.6)	1.45 (0.74–2.83)	-
Metabolic or endocrine disorder	4 (0.9)	3 (3.7)	3.99 (1.35–11.76)	4.22 (1.76–10.11)
Immune deficiency	57 (13.6)	18 (22.2)	1.81 (0.96–3.43)	1.91 (1.05–3.49)
Preterm birth	17 (4.1)	7 (8.6)	2.24 (1.26–3.95)	2.52 (1.41–4.49)

OR, odds ratio; IQR, interquartile range.

[a]Race/ethnic group was collected by study personnel based on auto reporting by the study participants. "Mixed race" refers to an individual of mixed European/Native heritage.

[b]Nutritional status was classified based on standard deviations for body mass index according to the World Health Organization into thinness (below −2 standard deviations [SD]), normal nutritional status (between −2 and +1 SD), overweight (between +1 and +2 SD) and obesity (above +2 SD). Body mass index was calculated as weight in kilograms divided by height in meters squared.

were less likely to require admission to critical care (35). Preferential distribution of ACE-2 receptors in the upper respiratory or intestinal epithelium (42) may explain the lower frequency of hospitalization in children with these clinical features. Between 9 and 10% of children in this series presented without fever or respiratory symptoms, or only developed diarrhea, findings that need to be considered when developing diagnostic algorithms, especially in settings with actively circulating virus.

Several laboratory findings have been associated with the severity of COVID-19 in adult patients (43). However, data on laboratory values as risk factors for the need for ICU admission in children are scarce. Of routinely collected data in children, only the C-reactive protein has been shown to be a predictor in one study (12), but not in others (44). Similar to our findings, leukocyte indices do not appear to be reliable indicators of disease severity in the pediatric population (45). We found that anemia was the only laboratory predictor for the risk of ICU admission. Whereas this finding has not been described in children, it has been documented as a significant risk factor in adults (46) and should be looked at in detail when assessing children with COVID-19. Like adults (47), bronchial wall thickening was associated with increased risk of ICU admission. Other more severe radiologic abnormalities often described in severe

TABLE 5 | Signs or symptoms, laboratory values and chest radiograph findings described or present on initial evaluation associated with intensive care, among pediatric patients hospitalized due to COVID-19 using multiple imputation chained equations for missing data.

Symptoms or signs - No. (%)	General ward (*n* = 419)	Pediatric intensive care unit (*n* = 81)	Univariable analysis OR (95% CI)	Multivariable analysis OR (95% CI)
Anosmia	3 (1.1)	3 (5.1)	-	-
Dysgeusia	6 (2.1)	2 (3.3)	-	-
Skin rash	21 (5.0)	2 (2.5)	0.48 (0.15–1.52)	-
Conjunctivitis	6 (1.4)	1 (1.2)	0.84 (0.07–10.47)	-
Abdominal pain	54 (13.7)	11 (14.9)	1.04 (0.36–2.97)	-
Fever	263 (62.9)	58 (71.6)	1.48 (1.06–2.08)	-
Cough	209 (50.1)	47 (58.0)	1.38 (0.74–2.54)	-
Pharyngitis	59 (14.2)	12 (14.8)	1.05 (0.37–3.00)	-
Rhinitis	111 (26.7)	22 (27.2)	1.02 (0.44–2.39)	-
Headache	39 (9.4)	9 (11.1)	1.21 (0.64–2.30)	-
Myalgia	22 (5.3)	8 (9.9)	1.96 (0.52–7.40)	-
Malaise	123 (29.6)	36 (44.4)	1.89 (0.76–4.71)	-
Diarrhea	61 (14.7)	19 (23.5)	1.78 (1.13–2.82)	2.23 (1.21–4.12)
Vomit	77 (18.6)	15 (18.5)	1.00 (0.59–1.71)	-
Dyspnea	137 (32.8)	53 (65.4)	3.89 (1.84–8.25)	-
Hypoxia	115 (27.5)	52 (64.2)	4.73 (2.08–10.80)	6.10 (2.40–15.50)
Altered mental status	13 (3.1)	26 (32.1)	14.50 (5.02–41.92)	5.38 (1.63–17.75)
Seizures	21 (5.0)	17 (21.2)	5.08 (3.30–7.82)	3.37 (1.80–6.32)
Dehydration	41 (9.9)	19 (23.7)	2.90 (1.12–7.50)	-
Shock	5 (1.2)	22 (27.2)	28.69 (11.92–69.04)	9.74 (2.44–38.89)
Onset of symptoms to, median (IQR)	1 (0–3)	1 (0–3)	0.99 (0.94–1.04)	-
Laboratory values				
Hemoglobin (g/dL)				
No.*	372	77		
Median (IQR)	12.3 (10.9–13.5)	11.4 (9.5–13.3)	0.90 (0.81–0.99)	
Anemia[a] No. (%)	106 (28.5)	34 (44.2)	1.98 (1.25–3.16)	2.34 (1.28–4.27)
Platelets (10^3 cells/uL)				
No.*	391	77	0.998 (0.997–0.999)	
Median (IQR)	299.0 (218–386)	276.0 (157.5–374.5)	2.46 (1.51–4.01)	
Thrombocytopenia[b] No. (%)	42 (10.7)	18 (23.4)		–
WBC (10^3 cells/uL)				
No.*	397	78		
Median (IQR)	10.0 (6.8–15.0)	12.2 (6.5–14.9)	1.00 (0.97–1.04)	
Neutrophils (10^3 cells/uL)				
No.*	397	76		
Median (IQR)	5.2 (2.5–9.3)	6.0 (3.0–9.3)	0.99 (0.94–1.05)	
Lymphocytes (10^3 cells/uL)				
No.*	394	76		
Median (IQR)	2.8 (1.6–4.6)	3.0 (0.9–5.2)	1.03 (0.96–1.09)	–
Neutrophil to lympho ratio				
No.*	393	76		
Median (IQR)	1.8 (0.7–4.3)	1.8 (0.8–4.8)	1.01 (0.95–1.07)	
Values > 5	86 (21.9)	18 (23.7)	1.10 (0.62–1.96)	–
C-reactive protein (mg/dL)				
No.*	332	70		
Median (IQR)	12.9 (4.2–39.5)	18.9 (7.0–102.7)	1.004 (1.002–1.006)	
Values > 50 mg/dL. No. (%)	70 (21.1)	23 (32.9)	1.88 (1.16–3.06)	–
Chest radiograph performed, No. (%)				
Yes	313 (74.7)	71 (87.65)		

(Continued)

TABLE 5 | Continued

Symptoms or signs - No. (%)	General ward ($n = 419$)	Pediatric intensive care unit ($n = 81$)	Univariable analysis OR (95% CI)	Multivariable analysis OR (95% CI)
Normal	194 (62.0)	29 (40.8)	Ref.	Ref.
Bronchial wall thickening	28 (8.9)	10 (14.1)	2.27 (0.97–5.28)	2.59 (1.15–5.84)
Interstitial infiltrates	55 (17.6)	16 (22.5)	2.08 (1.19–3.63)	1.01 (0.48–2.16)
Consolidation	23 (7.3)	8 (11.3)	2.53 (1.28–4.98)	1.10 (0.51–2.35)
Interstitial infiltrates + consolidation	13 (4.1)	8 (11.3)	4.32 (1.17–15.93)	1.14 (0.26–5.04)

WBC, white blood cell count.
[a]*Values < 11 g/dL.*
[b]*Platelets <150,000 × 10^3 cells/μL.*
[*]*Number of patients with available data.*

cases (48) were not independently associated with risk of ICU admission in this series, likely due to low number of cases.

Several studies have described risk factors for severe COVID-19 in high-income countries, but data is still scarce from LMIC. Our findings are similar to those from the UK cohort of children for admission to critical care (35). In both studies, children who required PICU were more likely to be of younger age, and had associated comorbidities. Studies from high-income countries have shown that adolescents and patients with elevated C-reactive protein have an increased risk for more severe outcomes (12, 16, 40, 49). These associations were not evident in our data, although different exposure categories and outcome measures may explain these discrepancies.

This study has several limitations. First, seven of the 14 reporting cities used a passive case-ascertainment method which probably introduced a selection bias. Thus, our study population does not represent the full spectrum of COVID-19, but rather children with more severe disease. This is unlikely to impact our results or conclusions given that our goal was to identify factors associated with hospital or ICU admission, and the multilevel regression model clustered by region. To further explore for selection bias, a sensitivity analysis including only children from the seven cities that used an active surveillance system was compared with the analysis that included all children. Factors associated with severity were similar in both analyses suggesting that the effect on our data was minimal.

Second, some relevant variables had missing data, which could reduce the statistical power of the study and produce biased estimates. We tried to overcome this limitation by using multiple imputation, allowing appropriate estimation of the underlying distribution of the data. Results from analyses using the complete dataset (including missing data) and the multiple imputation dataset were similar. Third, we excluded children with MIS-C, who represent one of the most severe clinical forms of pediatric post-infectious COVID-19. A separate full analysis of MIS-C children in Latin American children is ongoing by investigators from the REKAMLATINA network in a cohort of children admitted at several hospitals in the region (50). Finally, due to the multisite nature of the study, full standardization of criteria utilized by all managing clinicians for hospitalization or ICU care was not possible. Therefore, children's outcome disposition may differ by hospitals' capacity and census demands, notwithstanding the severity of the COVID-19 presentation. However, the need for ICU admission or oxygen is an objective measure for a more serious condition.

In conclusion, this evolving collaborative network allowed the collection of detailed data in one of the largest studies from a LMIC region to provide a first description of associated factors for hospital or ICU admission in developing countries. The demographic, clinical and laboratory parameters that were identified could help care providers in different settings (outpatient clinic, emergency room or general pediatric wards) to identify children at higher risk for a complicated disease course, and direct policy makers to prioritize pediatric subgroups for prevention and treatment strategies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and the registry and study protocol were reviewed and approved by the local Institutional Review Board of each participant center. The research was deemed of minimal risk because data was collected for routine clinical practice and the requirement for informed consent was waived. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

EL-M and EA: study conception and design. EL-M, GC-M, MB, DD, JT, RU-G, PL, RD, PP, JP, XN, CM, ML, GE, CD, KL, PQ, MR, JR-A, AE-V, MC, OM, EB, JC, AM, AJ-Z, LD, MM, and NG: data collection. EL-M, EC, and EA: analysis and interpretation of results. E-LM and EA: draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.868297/full#supplementary-material

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Effect of COVID-19 Pandemic on Well Child Care and Vaccination

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Objectives: The COVID-19 virus is highly contagious primarily via aerosol transmission and has a high mortality rate. On March 13, 2020, the United States declared a national emergency in response to the COVID-19 pandemic. This study aims to enumerate the effect of the pandemic on vaccination rates during the COVID-19 lockdown and the aftermath in pediatric patients aged 6weeks-6 years.

Study Design: A retrospective review of medical records was performed of missed well childcare visits at MetroHealth from March 1, 2020 to June 30, 2020. The sample size of 400 children aged 6 weeks to 6 years were randomly selected. Demographic data, number of calls made to attempt, scheduled WCC, no show rates for clinic appointments, number of missed WCC, location of MH facility, insurance type, vaccination status prior to the pandemic were collected. Statistical analysis was performed with SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

Results: From this descriptive study, we found that 43.5% of patients were not up to date on their childhood vaccination. The mean age was 24.38 months (SD 20.15). There were slightly more males (52.8%) in the study than females (47.3%) and most children were of African American descent. More than 50% of patients missed a scheduled well child appointment and 27% had a missed at least two consecutive appointments.

Conclusion: The COVID-19 pandemic has no doubt made a significant mark on health care; the effects would be both immediate and delayed, with vulnerable population being the most impacted. There is an urgent need to prevent a large-scale health disaster of catastrophic potential that could occur if an effective vaccination strategy is not implemented rapidly.

Keywords: COVID-19, pandemic, well child care visits, vaccination, children

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INTRODUCTION/BACKGROUND

The first U.S. cases of non-travel related COVID-19 were confirmed on February 26 and 28,2020. During this time, there was little known scientific information on its virulence and the aftereffects of the illness. The COVID-19 virus was proving to be highly contagious primarily via aerosol transmission and had a high mortality rate. On March 13, 2020, the United States declared a national emergency in response to the Coronavirus (COVID-19) pandemic.

The pandemic changed everyday life in many ways and had a profound effect on health care delivery in the United States. This effect on health care was global, as according to a WHO report published in August 2020, 90% of 105 countries reported at least some disruptions to essential health services, with routine immunization services, among the most frequently disrupted. (1, 2). The pediatric population though impacted, did not experience a high mortality rate. Families heeded warnings from public health officials and kept children inside and home from schools and daycares to limit exposure to the virus. However, strict adherence to home quarantine created a new problem that placed infants and children at a great disadvantage of missed evaluations by medical personnel (3, 4). In a bid to protect children, parents simply stopped showing up for routine well-child care and medical illnesses. This trend continued even when physicians' offices reopened for limited in-person visits. This created a situation where vaccines were deferred. The Boston Medical Center which serves about 14,000 vulnerable patients, 90% of whom are on public insurance, recorded an 80% decrease in clinic volume in March 2020. (3). This scenario was widespread across numerous pediatric practices across the country. Vaccines for Children program (VFC) provides vaccinations to children who are both underinsured and insured. Funding for the VFC program is approved by the Office of Management and Budget (OMB) and allocated through the Centers for Medicare & Medicaid Services (CMS) to the Centers for Disease Control and Prevention (CDC). Vaccines available through the VFC Program are those recommended by the Advisory Committee on Immunization Practices (ACIP)(5). VSD is a collaborative project between CDC's Immunization Safety Office and eight U.S. health care organizations serving publicly and privately insured patients.VSD indicated a notable decrease in orders for VFC-funded, ACIP-recommended vaccines, specifically non-influenza childhood vaccines and measles-containing vaccines during January 6, 2020- April 19, 2020, compared to January 7, 2019–April 21, 2019. (6). The decline began following the declaration of a national emergency by President Trump on March 13, 2020, similar declines in orders for other vaccines were also observed. (6). Worldwide, in a poll of 260 healthcare practitioners in May 2020, respondents in 53(85%) of 61 countries reported lower vaccine levels than those recorded in January and February 2020 (1, 7).

It is important to avoid a disruption in immunizations to prevent communities from vaccine-preventable diseases and outbreaks during the COVID-19 pandemic. Per Vogt et al. study, the results of a current national survey indicated that most VFC-enrolled practices were open and offering routine immunization services to all pediatric patients in May 2020 (8). There was a sharp decline in VFC vaccine orders beginning in March and continuing through April (6), orders during the second half of May 2020 and the first 3 weeks of June were relatively comparable to those from the same period in 2019), suggesting that the current immunization infrastructure can meet the expected need to provide vaccines that are overdue to many children. concerns about access to routine immunization services among certain populations of children, particularly those living in urban areas and the Northeast (8).

This descriptive study aims to enumerate the effect of the pandemic on vaccination rates during the COVID-19 lockdown and the aftermath in pediatric patients aged 6weeks-6 years receiving medical care at Metro Health System's outpatient pediatric primary care clinics from March 1 to October 30th, 2020. A list of missed scheduled well-child visits during a specified period was identified and a retrospective review of medical records was performed. Demographic, categorical, and continuous data were collected and analyzed as depicted below.

METHODS

Overview

A retrospective review of medical records was performed of missed well childcare visits (WCC) at Metro Health (MH) from March 1, 2020, to June 30, 2020. Among 9,594 children scheduled for WCC, approximately 3,680 missed WCC were identified in children aged 0-18 years. The sample size of 400 children aged 6 weeks to 6 years was randomly selected. Inclusion criteria were pediatric patients receiving medical care at MH hospital before

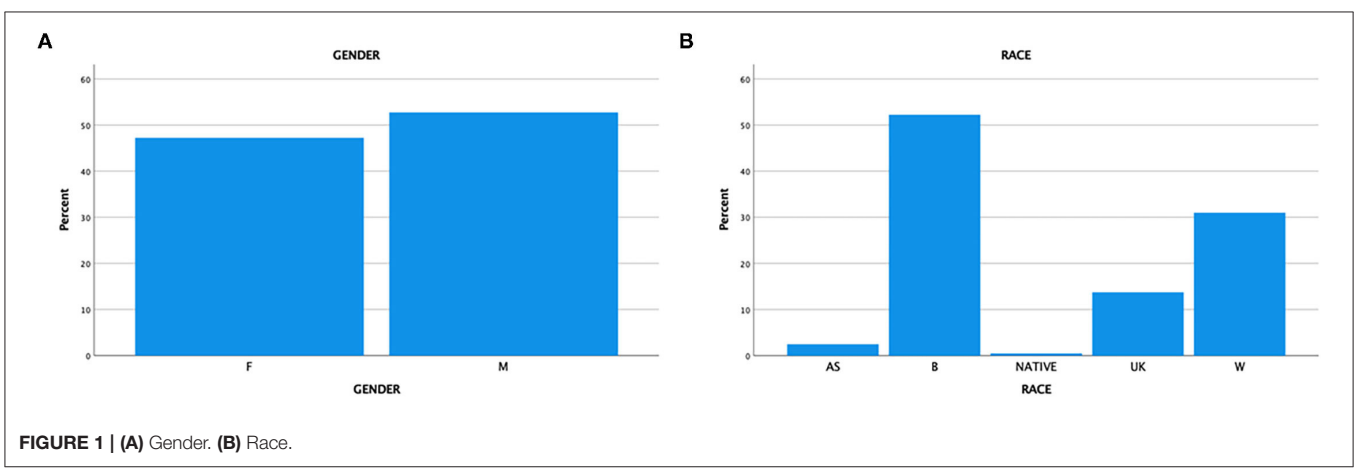


FIGURE 1 | (A) Gender. **(B)** Race.

COVID-19 and newborns who initiated care at MH facilities. Patients were excluded if they were >18 years of age, utilizing non-MH facilities for primary care, and did not establish care in Pediatric MH Clinics from March 2020 to June 2020. The primary endpoint was demonstrating the effect that COVID-19 had on childhood vaccination rates via missed clinic visits. Secondary endpoints included identifying locations with the highest number of missed visits and the vaccines/age group most impacted. Results will be utilized to map out a program intervention to increase pediatric childhood vaccination rates.

Data

Data were then extracted from clinic visits that occurred between March 1 and October 30, 2020. Delayed vaccination was defined as vaccines that were not administered due to a missed scheduled WCC. WCC was defined as an in-person clinical encounter for a routine physical, recommended annually for children aged above 3 years and more frequently for infants and children under 3 years of age.

Demographic data including age, gender, ethnicity, and zip codes were collected.

Continuous variables (number of calls made to attempt, scheduling WCC, no show rates for clinic appointments) and categorical variables [zip codes, number of missed WCC, location of MH facility, insurance status (YES/NO), insurance type, vaccination status before the pandemic—up to date (UTD) and not up to date (NUTD)] were collected. Statistical analysis was performed with SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Graphical and numerical summaries represented the data collected. Results are expressed as mean ± standard deviation (SD) for normally distributed variables. Chi-Square tests evaluated MH clinic location and zip code about vaccination status during COVID-19. All analysis was two-tailed, and p-values < 0.05 were considered statistically significant.

TABLE 1 | Descriptive statistics *demographics*.

Variables	Total $n = 100$
Gender	
Male	52.8%
Female	47.3%
Race	
Asian	2.5%
Black	52.3%
Native	0.5%
Unknown	13.8%
White	31%
Zip Code	
44101-44110	48%
44111-44120	19.5%
44121-44130	11.8%
44131-44140	13%
44141-44150	4.5%
44151 and greater	0.8%
44010-44100	2.5%
Insurance Status	
CareSource	63.7%
Molina	6%
UHC	7.5%
Buckeye	7.5%
Paramount medical	3.3%
Anthem blue	0.8%
Comm insurance	0.8%
Traditions medicare	1.0%
Medical mutual	2.0%
Medflex	0.3%
Aetna	0.5%
Cigna	0.3%
MH insurance	1.5%
Uninsured	4.3%
Dental C	0.5%
Medicaid	0.3%

RESULTS

Four hundred patients were randomly selected for the study. The mean age was 24.38 months (SD 20.15); a large SD indicates that most individuals' ages are spread out far from the mean. There were slightly more males (52.8%) in the study than females (47.3%) and most children were of African American descent (**Figures 1A,B**, **Table 1**). Most patients had public health insurance majorly, 4.3% of patients were uninsured; the near majority (48%) resided in the East Cleveland region (zip code 1; 44101–44110) (**Figures 2A,B**), which has a poverty rate of 37.51% (9).

Of the 16 MH pediatric clinic sites, the main campus (MHMC) was the site where most patients received care (33.5%), 12.5% received care at the Broadway center (**Figure 3**) and the remaining 15 sites were in the minority. Forty-four percent of patients were NUTD before the scheduled well-child visit in 2020 (**Figure 4A**). The outpatient service line placed priority in remainders being sent for scheduled visits; there were few documented calls to remind caregivers to schedule appointments, but this does not translate to the calls not being placed. More than 50% of patients missed a scheduled well-child appointment; 27% had missed at least two scheduled visits (**Figure 4B**). No show rates were a fixed value in electronic medical records (EMR) that did not specifically reflect the study period but rather the overall visits to the facility. The mode for no-show rates was 50%. MHMC clinic site had the greatest counts of missed visits and NUTD patients, however, there was an effort made by caregivers to attend visits as 63.2% of patients were seen after a missed visit. The vaccines that had the highest frequency of being missed were the DTaP while the most common vaccine combination missed was Hepatitis B/DTAP/IPV/PCV 13/Hib/Rotavirus (**Figures 5A,B**). Interestingly, few vaccination combinations were missed only once or twice, due to study

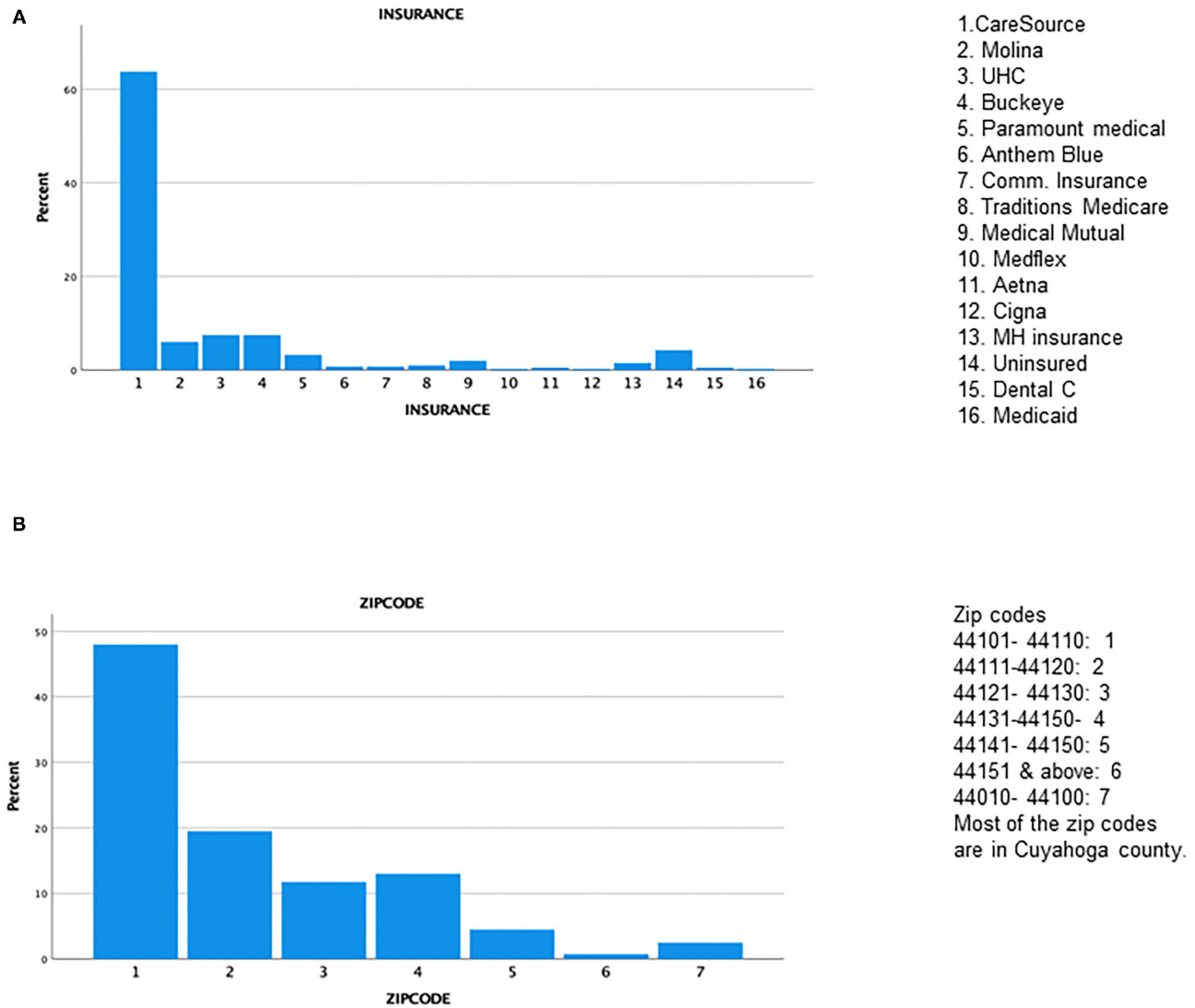
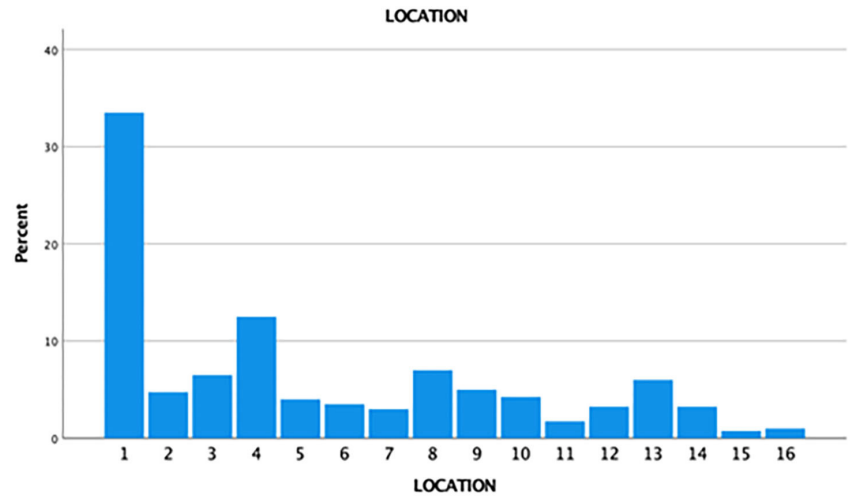


FIGURE 2 | (A) Insurance status. **(B)** Zipcode.

subjects being delayed probably even before the pandemic. Several combinations of the 2, 4, 6-month shots excluded the rotavirus, as the infant was probably beyond the maximum age it could be administered. There was a high frequency of missed optional vaccine hepatitis A although data collection did not distinguish between first and second dose, there was an anecdotal report of parents requesting deferment on the optional vaccine during the pandemic. The results reported a higher-than-expected number of patients that were NUTD in zip codes 1 (MHMC), 3 (Brooklyn), and 4 (Broadway), all areas with median household income lower than the state (Ohio) median income (9). Location 8 (Ohio City) had the highest divergence of the observed from the expected; the higher number of patients receiving care at this location were NUTD (19 patients) than expected (12.2 patients). This contrasted with the most common location for care (MHMC) having nearly equivocal data for observed and expected dichotomous data (NUTD: observed 59; Expected 58.3. UTD: Observed 75; Expected 75.5).

There was no correlation between vaccination status and location ($p = 0.370$), nor was there any correlation between vaccination status and zip code ($p = 0.122$) (**Table 2**). Previous vaccine status correlated with race; black populations were more likely to be NUTD on vaccinations ($p = 0.045$). There was no correlation between previous vaccine status (UTD/NUTD) and showing up for a clinic visit after a missed appointment ($p = 0.092$).



Location of Care
1. MHMC Peds, FP, Peds Hispanic
2. BCY Peds, Med-Peds
3. BR Peds, FP
4. BWY Peds, FP
5. PHE Peds
6. MBH Peds
7. CHE Peds
8. OHC Peds, Peds Hispanic
9. MCC Peds, Peds Hispanic
10. MCC Peds, Peds Hispanic
11. BRU Peds, FP
12. WCP Peds, FP
13. BF Peds
14. BCH Peds
15. BV Peds
16. JGS Med-Peds, FP

FIGURE 3 | Pediatric clinic locations.

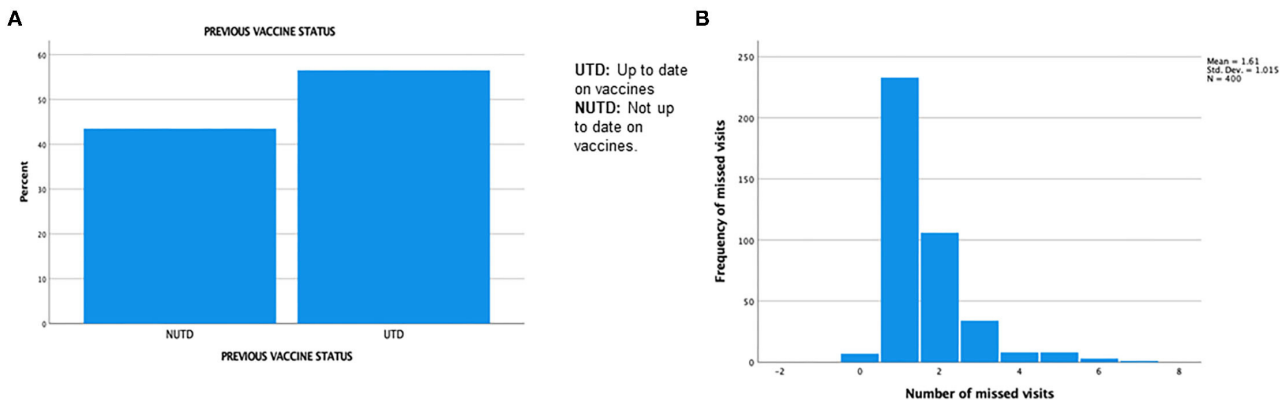


FIGURE 4 | (A) Previous vaccine status. **(B)** Number of missed clinic visits.

DISCUSSION

The COVID-19 pandemic has un-folded cracks existing in the healthcare industry. Disruptions to healthcare-associated with the COVID-19 pandemic have been well described (10–14). While it is encouraging that 56.5% of patients were UTD on childhood immunizations before the pandemic, it is worrisome that 43.5% were not. Well-child visits are settings in which childhood vaccinations are routinely administered and our study shows a reduction in scheduled visits which translates to interrupted schedules in vaccination of patients. Of note, no-shows were highest with areas with reduced median incomes suggesting the impact of social determinants of health. There was a paucity of documented efforts to contact patients to schedule WCC visits. Though we did not collect data on reasons for missed visits, we found in our clinical practice tele visits that most families communicated that they would not come to the facility due to the ongoing pandemic. Telemedicine though available during this period had the obvious limitation of inability to perform physical examinations or administer vaccines.

Our study mirrors findings abroad, as an international study showed a lower number of children received vaccination in 2020 compared with 2019 ranging from 50 to 85% depending on the individual vaccine analyzed (15). Interestingly, anecdotal reports from this international study noted there were no problems with vaccine supplies in the areas studied due to COVID-19

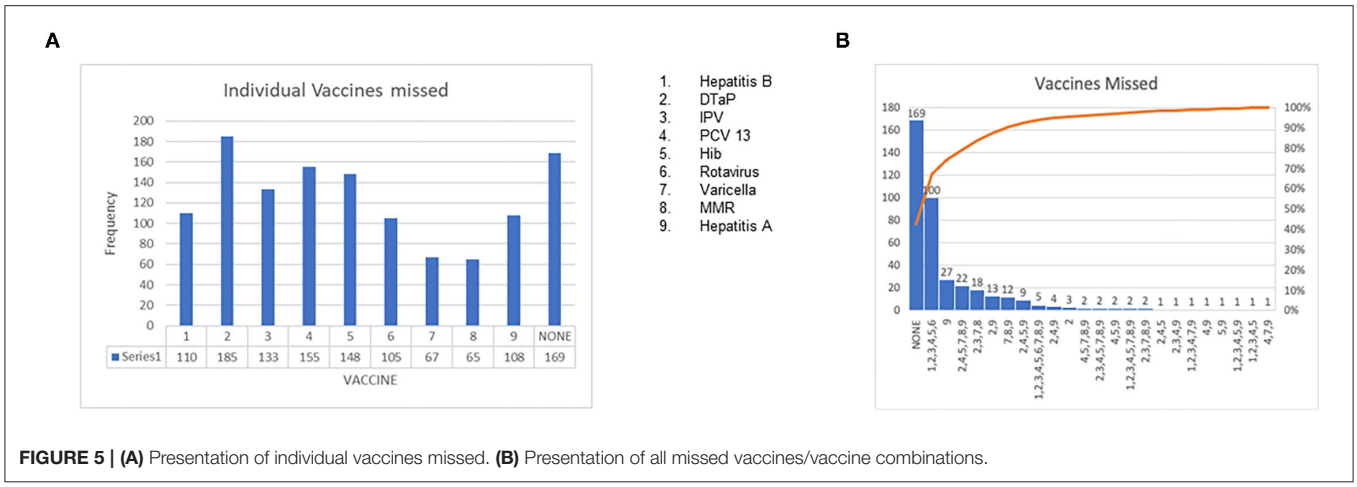


FIGURE 5 | (A) Presentation of individual vaccines missed. **(B)** Presentation of all missed vaccines/vaccine combinations.

TABLE 2 | Bivariate analysis-categorical outcomes (vaccination status).

Variable	Vaccination		
	UTD N = 56.5%	NUTD N = 43.5%	*P*-value
Location			
1	56%	44%	0.370
2	57.9%	42.1%	
3	53.8%	46.2%	
4	58%	42%	
5	68.8%	31.3%	
6	78.6%	21.4%	
7	50%	50%	
8	32.1%	67.9%	
9	55%	45%	
10	58.8%	41.2%	
11	85.7%	14.3%	
12	46.2%	53.8%	
13	66.7%	33.3%	
14	46.2%	53.8%	
15	66.7%	33.3%	
16	75%	25%	
Zip Code			
44101–44110	53.1%	46.9%	0.122
44111–44120	62.8%	37.2%	
44121–44130	55.3%	44.7%	
44131–44140	48.1%	51.9%	
44141–44150	77.8%	22.2%	
44151 and beyond	100%	0%	
44010–44100	56.5%	43.5%	

restrictions, hence the reduced vaccinations were not related to vaccine supplies (15).

Recommendations to increase vaccination status should include mandating calls by identified MH staff to caregivers so they can provide information on missed vaccine status and the importance of complying with clinic visits for vaccinations. During these calls, MH staff should discuss the safety precautions being taken by the facility to limit the risk of virus transmission. Mailed fliers, MyChart messages, and asking the patient/caregiver what mode of reminder works best for their lifestyle can be employed to remind caregivers of scheduled appointments.

Designing vaccination drives to occur at the most impacted locations in a publicly held facility with proper social distancing and sanitization, along with the engagement of community leaders is another strategy of increasing the vaccination rate. Expanding the vaccine drive-through program to occur at the campus that hosts the population with the lowest vaccination status and or sites where a large majority of patients receive care (MHMC), should be enacted. Providing incentives to caregivers that encourage compliance with WCC will be helpful.

Expanding clinic hours through offering evening and weekend clinics at select sites could be beneficial to the cause of catching up on immunizations. In addition, when schools reopen in person, implementing vaccination catch-up booths at schools, after prior consent from parents, will prove helpful for older children. There is a growing need to increase awareness among the public about the need to keep up with vaccinations, through social media and other networking platforms by trusted sources, to prevent the spread of misinformation, dispel myths and regain confidence in the health care system.

Though the majority were on public insurance, there has been monumental job loss resulting in loss of employer-based insurance, and this subset of patients impacted should be identified and offered free vaccination that could potentially be underwritten in cots by the local health department.

Finally, COVID-19 has no doubt made a significant mark on health care, the effects would be both immediate and delayed, with the vulnerable populations being the most impacted. This calls for well-coordinated action and engagement in increasing vaccination rates across various spheres. This study reports important information on vaccination and well-child evaluations during the pandemic in a medically underserved

population. Study limitations include possible selection bias and information bias, Unable to directly elicit reasons for missed visits, Study period not an extended duration, and the design method does not include ongoing/future data collection. Additionally, we were also unable to compare no-show rates before the pandemic to current no-show rates as it is only reported as an aggregate of scores by the electronic health records.

In summary, a pandemic is in season, but there is an urgent need to prevent a health disaster of catastrophic potential, which could occur if preventable diseases that are deceased by effective vaccination strategy develop rapidly due to a failure to strategize and increase vaccination rates during a pandemic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by MH IRB. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GO designed research. All listed authors contributed in retrieving data, analyzing data and manuscript writeup. All authors contributed to the article and approved the submitted version.

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Corrigendum: Effect of COVID-19 pandemic on well child care and vaccination

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CASE REPORT
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Case Report: Prolonged Neutropenia in Premature Monoamniotic Twins With SARS-CoV-2 Infection Acquired by Vertical Transmission

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Background: Vertical transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a highly debated topic in the current pandemic situation. Early neonatal SARS-CoV-2 infection is rare and generally mild. Long-term data describing symptoms after COVID-19 in premature neonates is scarce.

Case Presentation: Two premature, monoamniotic neonates were born by cesarean section to a mother 5 days after onset of symptomatic COVID-19. On day three of life both neonates developed hyperthermia, respiratory distress, and hematological changes, of which neutropenia persisted for over 40 days. Nasopharyngeal swabs for SARS-CoV-2 turned positive four days after delivery although the neonates were strictly isolated. Both neonates showed nearly identical time courses of ct values.

Conclusion: Our case report revealed prolonged low absolute neutrophil counts in two preterm neonates with symptomatic SARS-CoV-2 infection that is reasonably assumed to have been transmitted vertically *in utero*. After preterm delivery to a SARS-CoV-2 positive mother, testing for SARS-CoV-2 infection in neonates is crucial. Both neutropenia and lymphopenia should alert physicians to test for SARS-CoV-2 infection and also to follow the case.

Keywords: SARS-CoV-2, monoamniotic twin pregnancy, neutropenia, vertical transmision, prematurity

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INTRODUCTION

The numbers of SARS-CoV-2 infections during pregnancy are constantly increasing, thus raising concern of mother-to-fetus transmission. Current data suggest that the risk is low for neonates born to infected women to test positive for SARS-CoV-2 (1–3). Although only rarely reported, placental infection of SARS-CoV-2 appears to be possible (4). This case study summarizes the clinical course of two premature, monoamniotic neonates in whom symptomatic SARS-CoV-2 infection developed early after delivery by elective cesarean section at 32 weeks of gestation, suggesting *in utero* vertical transmission of SARS-CoV-2.

CASE REPORTS

A 33-year-old woman (gravida 2, para 1) was admitted to our hospital at 29 weeks and 6 days of gestation for surveillance owing to the high-risk constellation of a monoamniotic twin pregnancy.

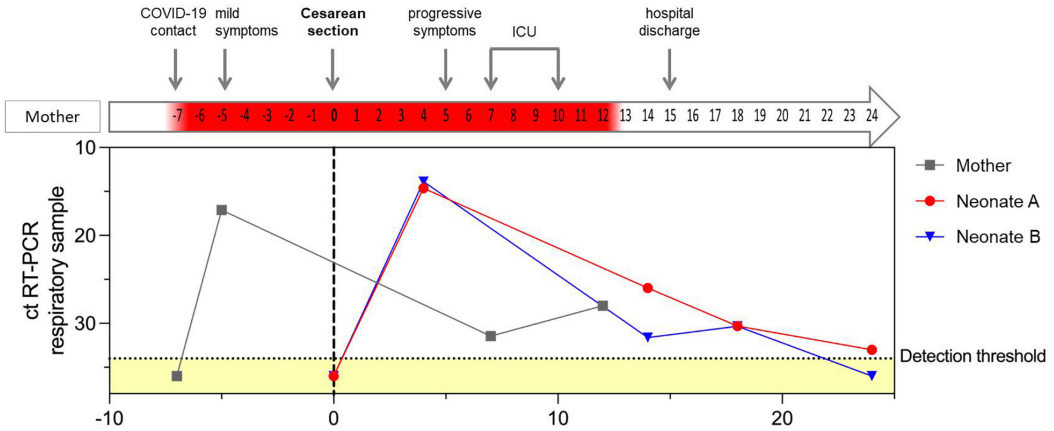

FIGURE 1 | RT-PCR cycle threshold values for SARS-CoV-2 in respiratory samples of the mother and both neonates in relation to maternal clinical course. The dotted horizontal line indicates the detection threshold set at 34, distinguishing between positive and negative (marked as yellow) RT-PCR results. Negative RT-PCR results are displayed as ct values of 36. Day of cesarean section is defined as day 0, represented with a gray, vertical, dotted line. Positive RT-CPR results of the mother's respiratory samples are highlighted in red in the arrow illustrating the maternal clinical course.

The pregnancy had gone well with regular prenatal care visits and normal ultrasound examinations. The patient had had close contact with a confirmed COVID-19 case, but tested negative for SARS-CoV-2 by RT-PCR. Two days later, however, she developed a mild cough and SARS-CoV-2 infection was confirmed by RT-PCR of her nasopharyngeal swab (**Figure 1**). The patient received symptomatic antitussive therapy without any need for additional oxygen supply. As aberrant patterns were intermittently detected by cardiotocography, the cesarean section was scheduled for 32 + 0 weeks of gestation. A full course of betamethasone for respiratory distress syndrome prophylaxis was administered. Elective cesarean section was then performed 5 days after onset of symptoms; the amniotic membranes were intact, there were no uterine contractions, and the patient was in full isolation under regional anesthesia.

Two female neonates were born: A weighing 1480 g and B weighing 1600 g. Cord blood analysis revealed normal pH values. At birth nasopharyngeal swabs of both neonates were tested negative for SARS-CoV-2 using RT-PCR. The neonates were placed in an incubator in an individual room of our neonatal intensive care unit (NICU) under contact and droplet isolation measures, according to current recommendations.

Shortly after birth, both neonates routinely received caffeine. Neonate A breathed spontaneously with FiO2 of 0.21. On day 3 of life, she developed fever up to 38°C and tachypnea. Therefore, treatment with ampicillin and gentamycin was started empirically for 3 days. She did not require respiratory support and fluctuating temperatures normalized within two days.

In contrast, neonate B required support *via* continuous positive airway pressure (CPAP) for respiratory distress during the first 10 h of life. Mild respiratory distress was confirmed by initial chest X-ray. Her condition remained stable until day 3 of life, when she developed secondary respiratory deterioration with dys- and tachypnea, whereupon CPAP was initiated followed by high-flow nasal cannula with respiratory improvement until day 8. Additionally, the frequency of apneas increased greatly, with repeated stimulation being needed despite additional caffeine doses. Subfebrile temperatures up to 37.6°C were observed after 78 h and stabilized spontaneously within two days.

After 91 h, SARS-CoV-2 infection was diagnosed in both neonates by RT-PCR in their nasopharyngeal swabs with low cycle threshold (ct) values and was confirmed by multiple consecutive RT-PCR tests (**Figure 1**). SARS-CoV-2 sequencing revealed the Delta variant in both neonates, as well as in the respiratory sample of the supposed index person of the mother (B1.617.2).

Laboratory measurements of both neonates always showed normal CRP levels, whereas absolute lymphocyte counts (< 1/nl) and total white blood cell counts (< 3/nl) were low on third day of life. Absolute neutrophil levels, however, were persistently low over months (**Figure 2**). Both neutropenia and lymphopenia preceded the SARS-CoV-2 positive swabs. Echocardiography and ultrasound of the brain were normal in both twins. Follow-up examination after four months revealed normal development of both premature neonates.

The mother remained hospitalized for 3 days and was discharged with a mild cough. After leaving the hospital, she developed a progressive cough and elevated temperature of 38.6°C. Due to increasing respiratory insufficiency and COVID-19 pneumonia she was transferred to our intensive care unit and received 40 L/min oxygen *via* a high-flow nasal cannula, piperacillin/tazobactam, caspofungin, remdesivir, and corticosteroids. Chest CT scan revealed bilateral ground-glass opacities. High-flow oxygen therapy was gradually reduced and after 4 days on the ICU she was transferred back to a regional hospital (**Figure 1**).

DISCUSSION

We present two cases of preterm monoamniotic neonates with a symptomatic SARS-CoV-2 infection, most probably vertically

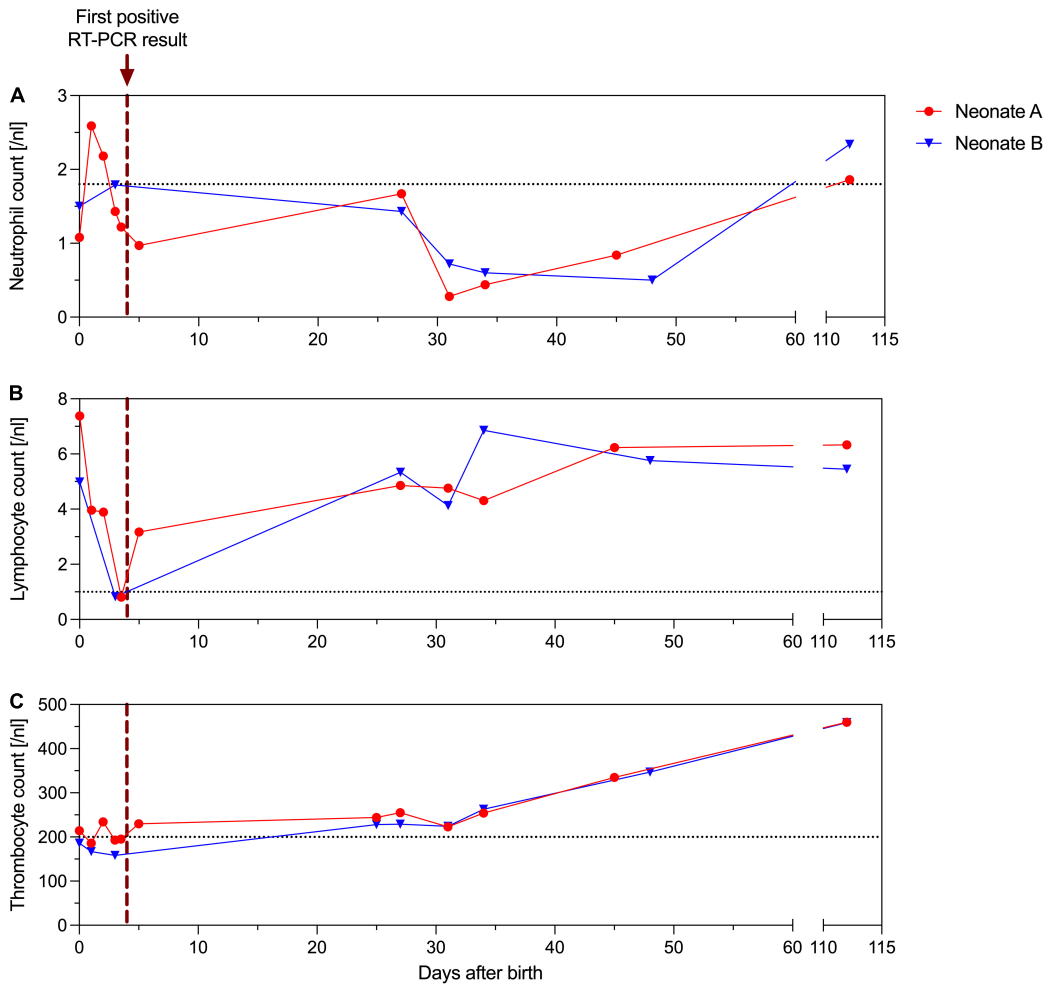

FIGURE 2 | Absolute neutrophil **(A)**, lymphocyte **(B)** and thrombocyte **(C)** counts [/nl] of neonate A (red) and B (blue). The purple dotted vertical line indicates the first positive RT-PCR result of the neonates' nasopharyngeal swab. The gray dotted horizontal line indicates lowest range of the reference interval.

transmitted *in utero via* the placenta. According to published reports, vertical transmission rates are low at about 3%, which is comparable to other congenital viral infections such as varicella or cytomegalovirus (5). COVID-19 symptoms in neonates are commonly mild or asymptomatic (6).

We are confident that we observed two actual symptomatic neonatal SARS-CoV-2 infections rather than contaminations, as a nearly identical time course for the ct values was observed in both twins. In our neonates, SARS-CoV-2 infection caused subfebrile temperatures and respiratory deterioration with severe central apneas from day 4 to 5 of life, delaying the hospital discharge of neonate B due to persistent hypopnea and apnea – persisting longer than would be expected as a result of the premature birth. Hyperthermia, respiratory distress, tachypnea, and apnea belong to the most common signs of COVID-19 in neonates (1, 6). Hematological abnormalities like low neutrophil and lymphocyte counts have already been reported (7–9). Here, these changes preceded the onset of symptoms and diagnosis of COVID-19. It is worth noting that the clinical course and the hematological changes in the two preterm neonates differed. Both preterm neonates were born with neutropenia, but only neonate A reached two average neutrophil counts before their first positive swab test, while neonate B did not reach average neutrophil counts for several months. In line with the hematological differences, the status of neonate B was inferior to that of neonate A and may reflect that their immunological response was slightly different at the beginning. Premature neonates and especially those with a disturbed immunological system at birth are naturally at high risk of respiratory illness and infections. Although neonatal SARS-CoV-2 infections are rare, newborns and especially preterm neonates require cautious observation, because they are more likely to suffer from longer intensive care and respiratory support after SARS-CoV-2 infection (1, 10). A novelty of our case is the prolonged period of low absolute neutrophil counts for several months after birth. The delayed recovery of the neutrophils may represent the unresolved inflammation in long COVID in

premature neonates (11). Ryan et al. recently demonstrated an ongoing immune dysregulation by profiling the immune cell populations in adults recovering from COVID-19 (12). Only little is known yet about immunological perturbations after early SARS-CoV-2 infection in premature infants.

Unlike other case reports describing vertical transmissions of SARS-CoV-2 (13), a unique feature of our case is the monoamniotic pregnancy, which offers a case-control-like situation with nearly identical courses of infection in both twins and suggests that exposure to SARS-CoV-2 took place at an identical time point under identical conditions – like *in utero*, sharing the identical placenta. Here, intraoperative and postnatal transmission appears very unlikely. The mother continuously wore a FFP2 mask, was not intubated, and had no contact with the neonates in the timeframe between birth and onset of symptoms, making droplet and aerosol contamination very unlikely. Postnatal infection seems nearly impossible considering the extensive quarantine measures and strict hygienic rules in our NICU. All staff members in contact with the neonates were asymptomatic during and after the delivery. In theory, *in utero* transmission of SARS-CoV-2 requires viremia and placental cell infection. Several reports of placental chronic intervillositis in SARS-CoV-2-infected women have been published (4, 14). Demirjian et al. described a comparable timing and course of vertical transmission with early neonatal SARS-CoV-2 infection with an initial negative test on the day of cesarean section that turned positive on the third day of life with proven maternal viremia on the day of delivery (13). Considering the short time interval between the onset of COVID-19 symptoms and delivery in our neonates, one may suggest that viremia was present in both mother and twins at the time of cesarean section. We speculate that the delayed detection of SARS-CoV-2 in respiratory samples can be attributed to the lack of viral colonization of respiratory mucosa *in utero*, which in turn is presumably due to the lack of breathing activity before delivery. That might suggest, too, that there was no viral contamination in the amniotic fluid. Our report has some limitations. First, this report is limited to a single case of monoamniotic twins. Second, blood samples were not analyzed for RT-PCR or antibody levels and no specific PCR testing of amniotic fluid, placenta, or umbilical cord was performed to definitively verify vertical transmission.

SARS-CoV-2 infection is associated with high risk of premature delivery (10, 15). This increased risk can be explained by either a critical or worsening condition of the infected mother or obstetric reasons like preeclampsia. Furthermore, SARS-CoV-2 infections were associated with higher rates of premature rupture of the membranes (15). The risk of fetal infections generally depends on gestational age at exposure. Placental SARS-CoV-2 infection requires the co-expression of the angiotensin-converting enzyme 2 (ACE-2) receptor and transmembrane protease serine 2 (TMPRSS2), which increases with advancing gestational age and may be directly linked to placenta susceptibility to SARS-CoV-2 infection (16). Especially if maternal infection occurs shortly before birth in late second or early third trimester, the immune system of the premature fetus may not be able to cope with the infection to the same extent compared to a fetus at term.

In conclusion, this case report provides deeper insights into the clinical course of vertically transmitted SARS-CoV-2 infection in premature infants. Early hematological changes should alert physicians to test for SARS-CoV-2 infection. These findings should contribute to better prevention and care among preterm neonates born to pregnant women with acute SARS-CoV-2 infection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AS and NK wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Clinical Characteristics, Laboratory Findings, Management, and Outcome of Severe Coronavirus Disease 2019 in Children at a Tertiary Care Center in Riyadh, Saudi Arabia: A Retrospective Study

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Background: Numerous studies worldwide have reported COVID-19 in children; however, the clinical symptoms and consequences of COVID-19 in children have only been reported in a few studies in Saudi and gulf region. Therefore, we aimed to investigate the clinical features and outcomes of COVID-19 infection in children and the therapeutic interventions used.

Methods: This retrospective cohort study included 96 patients with confirmed severe acute respiratory syndrome coronavirus 2 infection aged ≤14 years who were admitted to a tertiary governmental care hospital in Riyadh, Saudi Arabia between March 2020 and November 2020. Data on children with COVID-19, including demographics, comorbidities, symptoms, imaging and laboratory results, therapies, and clinical outcomes, were analyzed.

Results: Of 96 children admitted with a confirmed diagnosis of COVID-19, 63.8% were aged ≤ 3 years, 52.1% were male, 56.2% had an unknown source of infection, and 51% had no comorbidities. Most cases had severe infection (71.88%) as they required oxygen, 10.42% of whom were critical. The most common symptoms were respiratory-related (98%), and the common physical sign was fever (49%). High D-dimer (90.7%) and C-reactive protein (72.09%) levels were found in most cases. Oxygen (71.88%) was the most commonly used treatment. Most patients were discharged home and fully recovered (97.92%). We reported two deaths (2.08%).

Conclusions: Our findings showed that the majority of the admitted children with COVID-19 were ≤3 years of age (52.1%) and infected with an unknown source (56.2%).

Moreover, the majority of the cases had severe COVID-19 infection as they required oxygen (71.88%), although they had favorable outcomes. However, some cases were critical and resulted in death. Future studies will be crucial to better understand the disease spectrum and potential therapeutic options for COVID-19 in children.

Keywords: COVID-19, characteristics, management, outcome, children, coronavirus disease

INTRODUCTION

Numerous studies worldwide have reported COVID-19 with different variants such as omicron, delta, and SARS CoV-2 infection in children. China has published one of the first comprehensive studies, which included 728 patients with SARS CoV-2 infection variant (1). Almost 12% of the patients had no symptoms, 43% had mild symptoms, 40% had moderate symptoms, and 2.9% had severe symptoms or were in critical condition. Children <1 year of age accounted for a larger proportion of severe or critical cases (1). Similarly, one systemic review reported 7,780 pediatric patients with proven SARS CoV-2 infection. Fever and cough (59.1 and 55.9%, respectively) were the most common symptoms of COVID-19 in children, and only 19.3% of patients were asymptomatic (2). Children with comorbidities were the largest subset of COVID-19 cases. Most patients (88.9%) recovered, a minority of patients were admitted to the intensive care unit (ICU) (3.3%), and seven deaths were reported (0.09%) (2). Furthermore, according to a study on the clinical characteristics of COVID-19 in children, fever, cough, and gastrointestinal involvement were far more common in children than in adults (3, 4). The condition is mild for most children, according to these COVID-19 studies in children, with a vast number of children remaining asymptomatic. However, MIS-C represents a subpopulation of children who are more prone to acquiring major disorders related to COVID-19 (2, 5). In a recent study of MIS-C in children and adolescents, 186 cases were discovered across the United States. The individuals' average age was 8.3 years, and 75% of them had previously been healthy. Aneurysms in the coronary arteries were found in 8% of patients, and 40% of them had symptoms similar to KD (5). Additionally, a systematic review and meta-analysis study showed that a high proportion of children with MIS-C were admitted to the ICU (47.1%), and 4.8% of hospitalized children with MIS-C died (4). Moreover, a study in SA showed that five patients diagnosed with MIS-C-associated SARS CoV-2 infection with severe clinical presentation were admitted to the ICU, where they received intravenous immunoglobulin and inotropic support (6).

There are few studies in SA and the Gulf region, on the clinical characteristics and consequences of COVID-19 in children (7–14). However, COVID-19 predisposing factors affecting Saudi adults such as diabetes, obesity and vitamin D deficiency has been documented (8–10). In a study of SARS CoV-2 infection in SA, 4.8% of cases were in children aged ≤14 years (11). The clinical features and outcomes of these pediatric patients, on the other hand, were not documented (11). However, one of the initial studies from SA detailed the clinical aspects of COVID-19 in children, revealing that 54% of the cases were male, with an average age of 8.4 years, and 75% had normal white blood cell counts and eosinopenia (15). In another similar study in SA, children infected with SARS CoV-2 had a median age of almost 7 years, and the most prevalent symptom was fever (32.5%), followed by respiratory symptoms (21%), and gastrointestinal symptoms (10.3%) (6). Seven patients with COVID-19-related symptoms were admitted to the ICU, five of whom had multisystem inflammatory syndrome in childhood (MIS-C), one had Kawasaki disease (KD), and one had pneumonia (6).

In the other hand, the latest infectious variant is Omicron, which was found to be more transmissible and less pathogenic than previously circulating variants (16). On December 1, 2021, the Omicron variant was first clinically diagnosed in the United States, and it quickly spread (16). Despite the fact that Omicron had the most COVID-19 cases and hospitalizations during the pandemic, illness severity measures such as duration of stay, ICU admission, and mortality were lower than during past pandemic peaks (16). This highlights the need of national disaster preparation when critical care demands occur before the system becomes overloaded. In addition, staying up to date on COVID-19 immunizations and implementing other suggested COVID-19 preventative practices are crucial to preventing infections, serious illness, or death from COVID-19 among unvaccinated people (16). Health care workers (HCWs) in SA showed a high conviction in SARS-CoV-2 Omicron Variant infection prevention measures (17). Moreover, increase stress among HCWs in SA immediately after a new infectious threat emerges (18). The Omicron variant is causing a new wave of the COVID-19 outbreak over the world. The origin, pathogenicity, virulence, and immunogenic escape of this variation are all yet unknown (19).

There are limited data available on the characteristics of severe SARS CoV-2 infection of COVID-19 in children in SA and the Gulf region despite there are many descriptive studies in children have been performed internationally. Therefore, this study aimed to investigate the clinical features and outcomes of severe COVID-19 presentation in children and the therapeutic interventions used.

MATERIALS AND METHODS

Study Design and Setting

This retrospective cohort study was conducted at a tertiary governmental care hospital in Riyadh, SA. This colossal medical facility comprises four hospitals and four medical centers. The hospital has >200 beds covered by general pediatric, subspecialty, and critical care teams. The necessity for informed consent was waived because this was a retrospective study that used

data gathered in routine clinical practice between March 2020 and November 2020. The study was approved by the local Institutional Review Board on January 2020.

Patients and Testing

We retrospectively screened children aged ≤14 years with confirmed severe SARS CoV-2 infection who required oxygen as per Saudi Ministry of Health (SMOH) with signs and symptoms of respiratory tract infection, fever, headache, body ache, loss of taste or smell, chest pain, skin rashes, and gastrointestinal symptoms who were admitted to the tertiary governmental care hospital in Riyadh, SA between March 2020 and November 2020. The rationale behind choosing children aged ≤14 years as an inclusion criterion is the accepted age for treating pediatric patients in SA. Patients with insufficient data, those who went to the emergency for only a nasopharyngeal swab test, and those who had previously been admitted for the same reason were also excluded. Patients who require oxygen were constituted as severe COVID-19 infection.

To screen for an acute respiratory illness among the suspected cases and determine who required naso/oropharyngeal swab testing, we used the Saudi Center for Disease Prevention and Control "Weqaya" (12) and the Saudi Ministry of Health (SMOH) standard visual triage checklist (13). The checklist asses the exposure risk by multiple questions if the patient has history of travel abroad in the past 14 days, a contact with a confirmed case of SARS CoV-2 infection in the last 14 days prior to symptom onset, an exposure to camel or camel's products in the last 14 days prior to symptom onset, or working in a healthcare facility. Also, the checklist asses the clinical signs and symptoms as fever or history of fever, cough, shortness of breath, headache or sore throat, nausea or vomiting, diarrhea, and if he has any chronic disease. Any patient scored ≥ 4 will be assessed by physician, isolated in a respiratory room, and wear a mask.

To confirm that all study patients were positive for SARS CoV-2 infection, we performed a real-time reverse transcription-polymerase chain reaction test for the qualitative detection of nucleic acids from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from upper respiratory specimens, such as naso/oropharyngeal tissues. All patients provided nasopharyngeal swabs in a child-friendly setting. The patient was considered COVID-19 positive if the initial swab result was positive, or if it was negative at first but positive on a second test. The swab test was repeated soon after the initial results were negative if there was a high clinical suspicion of COVID-19 or a risk of false-negative results due to technological mistakes during sample processing. Some patients had multiple positive swab samples during their stay, as required by the responsible team in accordance with the center's local procedure and revisions in the SMOH's recommendations, which was altered over time based on the most recently published data.

Data Collection

Data was gathered from patient's electronic health records. Patient demographics (age, sex, and nationality), length of hospital stay, duration of symptoms, potential cause of infection, need for pediatric intensive care unit (PICU) admission, length

TABLE 1 | Patients demographics and disease characteristics associated with the outcome of children admitted with confirmed COVID-19 infection ($n = 96$).

Variables		Total population $n = 96$ (%)	Outcome	
			Full recovery (%)	Death (%)
			$n = 94$ (97.92%)	$n = 2$ (2.08%)
Age	≤3	60 (63.8%)	60 (63.8%)	0 (0.0%)
	>3	36 (36.20%)	34 (36.2%)	2 (100.0%)
Sex	Male	50 (52.1%)	48 (51.1%)	2 (100.0%)
	Female	46 (47.9%)	46 (48.9%)	0 (0.0%)
Nationality	Saudi	93 (96.9%)	91 (96.8%)	2 (100.0%)
	Non- Saudi	3 (3.1%)	3 (3.2%)	0 (0.0%)
Length of hospital Stay	≤10	70 (74.5%)	70 (74.5%)	0 (0.0%)
	>10	26 (25.5%)	24 (25.5%)	2 (100.0%)
Duration of symptoms	≤3	68 (70.83%)	67 (71.3%)	1 (50.0%)
	>3	28 (29.17%)	27 (28.7%)	1 (50.0%)
Possible source of infection	Contact COVID-19 Case	40 (41.67%)	39 (41.5%)	1 (50.0%)
	Travel to endemic area	2 (2.08%)	2 (2.1%)	0 (0.0%)
	Unknown	54 (56.25%)	53 (56.4%)	1 (50.0%)
Comorbidities	Yes	47 (49.0%)	45 (47.9%)	2 (100.0%)
	No	49 (51.0%)	49 (52.1%)	0 (0.0%)
***Complications**	Secondary Pneumonia	10 (10.4%)	9 (90.0%)	1 (10.0%)
	ARDS	2 (2.1%)	1 (50%)	1 (50.0%)
	Multiorgan failure	4 (4.2%)	4 (100.0%)	0 (0.0%)
	CNS complications	3 (3.1%)	2 (66.7%)	1 (33.3%)
	No complications	82 (80.2%)	81 (98.8%)	1 (1.2%)
***Treatments**	Oxygen	69 (71.88%)	67 (69.8%)	2 (100.0%)
	Steroid	13 (13.54%)	12 (12.5%)	1 (50.0%)
	Bronchodilator	10 (10.42%)	10 (10.4%)	0 (0.0%)
	Hydroxychloroquine	10 (10.42%)	10 (10.4%)	0 (0.0%)

(Continued)

TABLE 1 | Continued

Variables		Total population $n = 96$ (%)	Outcome	
			Full recovery (%)	Death (%)
			$n = 94$ (97.92%)	$n = 2$ (2.08%)
	Antiviral	6 (6.25%)	5 (5.2%)	1 (50.0%)
	Antibacterial	47 (48.96%)	45 (46.9%)	2 (100.0%)
Discharge	Home	94 (97.92%)	94 (100%)	0 (0.0%)
	Dead	2 (2.08%)	0 (0.0%)	2 (100.0%)

*Percentage does not add up to 100 because more than one element was used.

of stay in the PICU, need for intubation, presenting symptoms and physical signs, and associated comorbidities were all collected. We also considered the possibility of multiorgan failure, acute respiratory disease syndrome, pneumonia, or central nervous system complications caused by COVID-19. The degree of respiratory distress, chest radiography results, and the need for oxygen therapy and/or mechanical ventilation were also considered.

Detailed demographic and clinical data, such as presenting signs and symptoms, length of PICU stay, need for mechanical ventilation, and electrocardiogram (ECG) abnormalities, were collected for any patient who required intensive care admission. Laboratory findings were reported, and the associated hematological abnormalities were assessed, including significant anemia, leukopenia, leukocytosis, neutropenia or neutrophilia, and thrombocytopenia. Other reported laboratory abnormalities included high liver function test results and lactic acid dehydrogenase and D-dimer levels. High acute-phase reactants [C-reactive protein (CRP) and ferritin] and evidence of any related infection (in the blood, urine, and tracheal aspirate) were highlighted. In addition, we recorded any viral respiratory infection that may be present.

Moreover, during the study period, we encountered some cases that fulfilled the MIS-C or pediatric multisystem inflammatory syndrome criteria as set by the Center of Disease Control and Prevention (14), Royal College of Pediatrics and Child Health (20), and the World Health Organization (21); thus, these were included in the study. We recorded their demographic data, presenting symptoms and signs, and severity of respiratory disease using their laboratory findings and outcomes.

In the pediatric age group, we referred to the most used drugs as active COVID-19 therapy. Patients who had completed their hospitalization by the conclusion of the study period, including those who were discharged home or died, had their clinical outcomes recorded. Clinical outcome indicators included the full recovery rate, death rate, length of hospital stay, PICU admission, and the necessity for invasive therapy such as mechanical ventilation.

Statistical Analysis

It is a descriptive analysis so no relation or association were tested. Continuous variables are presented as mean ± standard deviation, and categorical variables are presented as frequencies and percentages. Statistical analysis was conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA).

Sample Size

Considering the novelty of the diseases at the time no parameters were used in sample size calculation and opted instead to include all patients who meet the inclusion criteria.

RESULTS

A 96 children admitted with a confirmed diagnosis of COVID-19 infection, of whom 63.8% were aged ≤ 3 years, 52.1% were male, 96.9% were Saudi, 56.2% had an unknown source of infection to SARS CoV-2, 51% did not have any comorbidities, 74.5% were hospitalized ≤ 10 days due to other comorbidities, and 70.83% had symptoms lasting for ≤3 days (**Table 1**). Moreover, as per the SMOH protocol, the majority of patients had severe COVID-19 (71.88%) as they required oxygen, 10.42% of whom were critical, and a minority of the cases had moderate COVID-19 (28.12%). Furthermore, most of the children did not develop any complications (80.2%), and oxygen (71.88%) was the most commonly used treatment, followed by antibacterial antibiotics (48.96%). The most common complications associated with COVID-19 were secondary pneumonia (10.4%), followed by multiorgan failure (4.2%), and central nervous system complications (3.1%). Most patients were discharged home (97.92%) and fully recovered (97.92%). Demographic data and disease characteristics are presented in **Table 1**.

The presenting symptoms and physical signs of children admitted with a confirmed diagnosis of COVID-19 were investigated. The most common presenting symptoms were respiratory symptoms (98%), followed by fever (75%) and gastrointestinal symptoms (50%); the remaining symptoms were present in <50% of patients (**Figure 1A**). CNS symptoms as abnormal movement and decrease level of consciousness were found in 16% of the patients. Fever was the most reported physical sign (49%), followed by dehydration (25%), tachycardia (17.7%), and respiratory signs (10.4%), while the other signs were present in <10% of the patients (**Figure 1B**).

Of the 96 children admitted, 10 (10.42%) were critical and required PICU care, as shown in **Table 2**. Most of them required hospitalization for >10 days (60%), but the mean PICU length of stay was ≤4 days (70%). The minority of patients admitted to the PICU required intubation (20%). Most patients had COVID-19 from an unknown source (80%) and had comorbidities (70%). Abnormal physical signs such as dehydration (70%), tachycardia (50%), and respiratory signs (40%) were more common in children who required admission to the PICU than in those who did not. Pneumonia (40%)

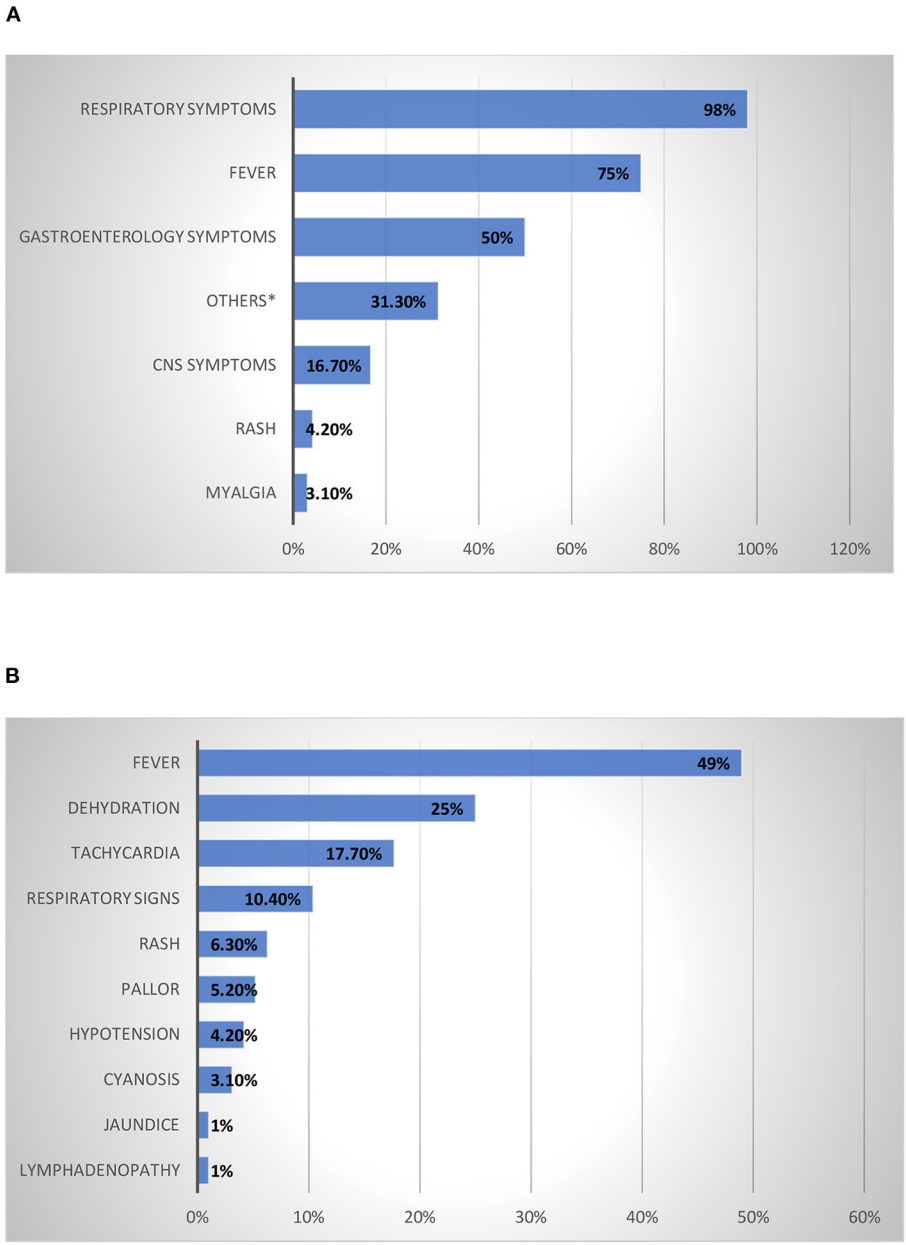

FIGURE 1 | Presenting Symptoms and Physical Signs of Children Admitted with A Confirmed Diagnosis Of COVID-19. **(A)** Presenting symptoms of children admitted with a confirmed diagnosis of COVID-19 infection. *Decrease activity and decrease appetite. **(B)** Physical signs of children admitted with confirmed diagnosis of COVID-19 infection.

followed by central nervous system complications (30%) were the most common complications. All patients were treated with oxygen (100%), followed by antibacterial antibiotics (80%) and steroids (40%). Most patients fully recovered (90%) and were discharged home (90%). We report one patient died in the PICU.

The laboratory results and imaging findings were assessed, and their correlations with PICU and ward admissions are shown in **Table 3**. Among the patients admitted to the regular ward, 86 (89.58%) tended to have the following findings: normal ECG findings, 83 (96.5%); normal chest X-ray findings, 72 (83.7%); abnormal chest computed tomography findings, 2 (2.3%); normal white blood cell count, 55 (64%); normal neutrophil count, 61 (70.9%); normal hemoglobin level, 59 (68.6%); normal lymphocyte count, 46 (53.49%); normal alanine aminotransferase (ALT) level, 81 (94.19%); normal aspartate aminotransferase (AST) level, 81 (94.19%); normal creatinine level, 49 (56.98%); normal ferritin level, 65 (75.58%); high D-dimer level, 78 (90.7%); high CRP level, 62 (72.09%); and adenovirus-positive result, 1 (1.16%). Those with positive bacteria in the blood (3 [30%]),

TABLE 2 | Patients demographics and disease characteristics with PICU vs. regular ward admission of children admitted with confirmed COVID-19 infection ($n = 96$).

Variables		Total population $n = 96$ (%)	Admission	
			PICU%	Ward%
			$n = 10$ (10.42%)	$n = 86$ (89.58%)
Age	≤3	60 (63.8%)	5 (50.0%)	55 (64.0%)
	>3	36 (36.20%)	5 (50.0%)	31 (36.0%)
Sex	Male	50 (52.1%)	8 (80.0%)	42 (48.8%)
	Female	46 (47.9%)	2 (20.0%)	(44 51.2%)
Nationality	Saudi	93 (96.9%)	10 (100.0%)	83 (96.5%)
	Non-Saudi	3 (3.1%)	0 (0.0%)	3 (3.5%)
Length of hospital stay	≤10	70 (74.5%)	4 (40.0%)	66 (76.7%)
	>10	26 (25.5%)	6 (60.0%)	20 (23.3%)
Pediatric intensive care unit length of stay	≤ 4	7 (70.0%)	7 (70.0%)	
	>4	3 (30.0%)	3 (30.0%)	
Intubation	Yes	2 (2.08%)	2 (20.0%)	
	No	94 (97.92%)	8 (80.0%)	
Duration of symptoms	≤3	68 (70.83%)	5 (50.0%)	63 (73.3%)
	> 3	28 (29.17%)	5 (50.0%)	23 (26.7%)
Possible source of infection	Contact COVID-19 Case	40 (41.67%)	2 (20.0%)	38 (44.2%)
	Travel to Endemic Area	2 (2.08%)	0 (0.0%)	2 (2.3%)
	Unknown	54 (56.25%)	8 (80.0%)	46 (53.5%)
Comorbidities	Yes	47 (49.0%)	7 (70.0%)	40 (46.5%)
	No	49 (51.0%)	3 (30.0%)	46 (53.5%)
***Signs**	Cyanosis	3 (3.1%)	0 (0.0%)	3 (3.5%)
	Fever	47 (49.0%)	8 (80.0%)	39 (45.3%)
	Dehydration	24 (25.0%)	7 (70.0%)	17 (19.8%)
	Hypotension	4 (4.2%)	1 (10.0%)	3 (3.5%)
	Pallor	5 (5.2%)	1 (10.0%)	4 (4.7%)
	Tachycardia	17 (17.7%)	5 (50.0%)	12 (14.0%)
	Respiratory Signs	10 (10.40%)	4 (40.0%)	6 (7.0%)
	Lymphadenopathy	1 (1.0%)	0 (0.0%)	1 (1.2%)
	Jaundice	1 (1.0%)	0 (0.0%)	1 (1.2%)
	Rashes	6 (6.3%)	0 (0.0%)	6 (7.0%)
***Complications**	Secondary Pneumonia	10 (10.4%)	4 (40.0%)	6 (6.98%)
	ARDS	2 (2.1%)	2 (20.0%)	0 (0.0%)
	Multiorgan Failure	4 (4.2%)	1 (10.0%)	3 (3.5%)
	CNS Complications	3 (3.1%)	3 (30.0%)	0 (0.0%)
	No Complications	82 (80.2%)	4 (40.0%)	78 (90.7%)
***Treatments**	Oxygen	69 (71.88%)	10 (100.0%)	59 (68.6%)
	Steroids	13 (13.54%)	4 (40.0%)	9 (10.5%)
	Bronchodilator	10 (10.42%)	3 (30.0%)	7 (8.1%)
	Hydroxychloroquine	10 (10.42%)	3 (30.0%)	7 (8.1%)
	Antiviral	6 (6.25%)	0 (0.0%)	6 (7.0%)
	Antibacterial	47 (48.96%)	8 (80.0%)	39 (45.3%)
Outcome	Full recovery	94 (97.92%)	9 (90%)	85 (98.83%)
	Death	2 (2.08%)	1 (10%)	1 (1.17%)
Discharge	Home	94 (97.92%)	9 (90%)	85 (98.84%)
	Dead	2 (2.08%)	1 (10%)	1 (1.16%)

Percentage does not add up to 100 because more than one element was used.

urine (3 [30%]), or tracheal aspirate [1 (10%)] were admitted to the PICU.

Among the 96 children admitted with confirmed COVID-19 infection, four were found to have MIS-C. The majority of the children who had MIS-C were aged >3 years (75%), all patients were Saudi, stayed for ≤10 days in the hospital,

TABLE 3 | Labs and images associated with pediatric intensive care unit vs. regular ward admission of children admitted with confirmed COVID-19 infection ($n = 96$).

Variables			Total population $n = 96$ (%)	Admission	
				Pediatric Intensive Care Unit %	Ward %
				$n = 10$ (10.42%)	$n = 86$ (89.58%)
Imaging	Electrocardiogram	Normal	92 (95.8%)	9 (90.0%)	83 (96.5%)
		Abnormal	2 (2.1%)	0 (0.0%)	2 (2.3%)
		Not Done	2 (2.1%)	1 (10.0%)	1 (1.2%)
	Chest X-ray	Normal	76 (79.2%)	4 (40.0%)	72 (83.7%)
		Abnormal	20 (20.8%)	6 (60.0%)	14 (16.3%)
	Chest computed tomography scan	Normal	3 (3.1%)	0 (0.0%)	3 (3.5%)
		Abnormal	3 (3.1%)	1 (10.0%)	2 (2.3%)
		Not Done	90 (93.8%)	9 (90.0%)	81 (94.2%)
Laboratory	White blood cells	Normal	61 (63.54%)	6 (60.0%)	55 (64.0%)
		High	27 (28.13%)	4 (40.0%)	23 (26.7%)
		Low	8 (8.33%)	0 (0.0%)	8 (9.3%)
	Neutrophils	Normal	67 (69.8%)	6 (60.0%)	61 (70.9%)
		High	22 (22.9%)	4 (40.0%)	18 (20.9%)
		Low	7 (7.3%)	0 (0.0%)	7 (8.1%)
	Hemoglobin	Normal	65 (67.7%)	6 (60.0%)	59 (68.6%)
		High	1 (1.0%)	0 (0.0%)	1 (1.2%)
		Low	30 (31.3%)	4 (40.0%)	26 (30.2%)
	Lymphocytes	Normal	51 (53.1%)	5 (50.0%)	46 (53.49%)
		High	27 (28.1%)	4 (40.0%)	23 (26.74%)
		Low	18 (18.8%)	1 (10.0%)	17 (19.77%)
	Alanine aminotransferase	Normal	90 (93.8%)	9 (90.0%)	81 (94.19%)
		High	6 (6.2%)	1 (10.0%)	5 (5.81%)
	Aspartate aminotransferase	Normal	89 (92.71%)	8 (80.0%)	81 (94.19%)
		High	6 (6.25%)	2 (20.0%)	4 (4.65%)
		Low	1 (1.04%)	0 (0.0%)	1 (1.16%)
	Creatinine	Normal	54 (56.25%)	5 (50.0%)	49 (56.98%)
		High	13 (13.54%)	2 (20.0%)	11 (12.79%)
		Low	29 (30.21%)	3 (30.0%)	26 (30.23%)
	Ferritin	Normal	73 (76.04%)	8 (80.0%)	65 (75.58%)
		High	18 (18.75%)	2 (20.0%)	16 (18.60%)
		Low	5 (5.21%)	0 (0.0%)	5 (5.81%)
	D.Dimer	Normal	7 (7.29%)	0 (0.0%)	7 (8.14%)
		High	88 (91.67%)	10 (100.0%)	78 (90.70%)
		Low	1 (1.04%)	0 (0.0%)	1 (1.16%)
	C-Reactive protein	Normal	7 (7.29%)	1 (10.0%)	6 (6.98%)
		High	69 (71.88%)	7 (70.0%)	62 (72.09%)
		Low	20 (20.83%)	2 (20.0%)	18 (20.9%)
	*Positive bacteria	Blood	5 (5.21%)	3 (30%)	2 (2.32%)
		Urine	4 (4.17%)	3 (30%)	1 (1.16%)
		Tracheal Aspirate	1 (1.04%)	1 (10%)	0 (0.0%)
Viral respiratory					
	Respiratory syncytial virus	Negative	23 (23.96%)	3 (30%)	20 (23.26%)
		Not done	73 (76.04%)	7 (70%)	66 (76.74%)
	Adeno virus	Negative	1 (1.04%)	3 (30%)	19 (22.1%)
		Positive	22 (22.92%)	0 (0.0%)	1 (1.16%)
		Not done	73 (76.04%)	7 (70%)	66 (76.74%)

(Continued)

TABLE 3 | Continued

Variables			Total population $n = 96$ (%)	Admission	
				Pediatric Intensive Care Unit %	Ward %
				$n = 10$ (10.42%)	$n = 86$ (89.58%)
	Para influenza	Negative	23 (23.96%)	3 (30%)	20 (23.26%)
		Not done	73 (76.04%)	7 (70%)	66 (76.74%)
	Influenza	Negative	23 (23.96%)	3 (30%)	20 (23.26%)
		Not done	73 (76.04%)	7 (70%)	66 (76.74%)

*Percentage does not add up to 100 because more than one element was used.

experienced symptoms for >3 days, and had no comorbidities. Fever (100%), diarrhea and vomiting (75%), and abdominal pain with decreased activity and appetite were the most prevalent presenting symptoms (50%). The most common abnormal physical signs in individuals with MIS-C were fever (100%), rash (50%), tachycardia (50%), hypotension (50%), and dehydration (50%). All patients with MISC developed multiple organ failure and received antibacterial treatment, and most of them received oxygen (50%). Generally, these patients did not require PICU admission (75%); all of them fully recovered in <10 days (100%) and were discharged home (100%).

Out of the 96 admitted children with confirmed SARS CoV-2 infection, we reported two deaths (2.08%), one in the ward due to respiratory cause and the other in the PICU due to complications of the disease. The two cases were aged >3 years old (100%), male (100%), Saudi (100%), stayed for >10 days in the hospital (100%) (**Tables 1**, **2**). One of the cases infected due to contact with COVID-19 infection and the other infected with unknown source. In addition, both cases known to have comorbidities as heart disease, renal disease, and neuromuscular disease. Fever and sore throat associated with cough, shortness of breath, and altered level of consciousness were commonly presented in both cases. Moreover, one of the cases developed complications of COVID-19 infection as secondary pneumonia, acute respiratory distress syndrome, and central nervous system complications whereas, the other case has no complications. Anemia, high creatinine, and high D.dimer were found in the both cases yet, one of them has positive blood culture. Although, both cases treated with oxygen, and antibacterial. However, one of the cases also treated with antiviral and steroids.

DISCUSSION

This is one of the retrospective studies in SA on the clinical features of pediatric patients with COVID-19. Studies published in late 2020 and 2021 were conducted in the SA and Gulf regions (5, 15, 22–24). However, three previous retrospective studies were published early in 2021: one retrospective study included 62 children from a single center in Jeddah, SA (25), the other conducted in a tertiary hospital in Riyadh included 742 pediatric inpatients and outpatients (6), and the last one conducted in three tertiary academic hospitals in SA enrolled 88 hospitalized children, the majority of whom had mild to moderate diseases (7). All the studies demonstrated that most patients had mild to moderate disease, and this may be due to the strict protocol endorsed by the SMOH (6, 7, 25). On the other hand, our study showed that the majority of hospitalized pediatric patients had severe COVID-19 infection according to the SMOH definition by requiring oxygen (71.88%). Fever and respiratory symptoms were also shown to be the most common clinical characteristics of COVID19 in children. Infants were more likely than other age groups to experience dyspnea. There have also been reports of gastrointestinal symptoms such as diarrhea and vomiting. Multiple studies have produced similar results (6, 7, 25).

Children with COVID-19 have been reported to more often have mild clinical symptoms than adults (26–28). In a review of studies regarding COVID-19 in children in SA and worldwide, most children with COVID-19 had mild symptoms, a good prognosis, and recovered within 1–2 weeks (6, 7, 25, 29). Our retrospective review showed that most of the patients had severe symptoms since oxygen was required in almost 71.88% of the cases, and they recovered within ≤3 days with good prognosis. Xiaoxia et al. (30) provided a summary of 171 confirmed pediatric cases in China, showing that most infected children had a mild clinical course, and asymptomatic infections were common (15.8%). There was one death involving a 10-month-old child with intussusception and multiorgan failure (30). In a large retrospective cohort study of children with COVID-19 in China, 4 and 51% of the patients were diagnosed without any symptoms or with mild symptoms, respectively, and only one child was reported to have died (1). A small percentage of patients in our study experienced complications, such as secondary pneumonia (10.4%) or acute respiratory distress syndrome (2.1%). Furthermore, the prevalence of severe and critical cases among infants (63.8%) was higher than in other age groups. Another retrospective study reported two critical pediatric patients aged approximately 1 year who required invasive mechanical ventilation, corticosteroids, and immunoglobulin therapy (31). However, our study found that only a minority of patients (10.42%) required PICU care, and two patients died. This shows that pediatric patients experience less severe illnesses than adults; nevertheless, young children, particularly infants, might have higher risk of sever COVID-19 infection than older children (1). One report from the United States (32) demonstrated that 73% of pediatric patients had symptoms of fever, cough, or shortness of breath as compared with 93% of adults. Between 5.7 and 20% of pediatric patients were reported to be hospitalized, with 0.58–2.0% admitted to the ICU. Two deaths were reported in this cohort of pediatric patients. Similar results have been reported in multiple studies (6, 7, 25). These findings show that children experience respiratory symptoms less frequently than

adults. Although the majority of pediatric patients with COVID-19 did not have a severe illness, a serious COVID-19 could lead to ICU admission and even death in children.

The main radiological features in pediatric patients with COVID-19 have been reported to be subpleural ground-glass opacities and consolidations with surrounding halo signs, suggestive of pneumonia (28, 33). The majority of children with these findings were asymptomatic or had only minor symptoms. Additionally, Qiu et al. (26) reported that the prevalence of pneumonia with COVID-19 (53%) was higher than that with H1N1 influenza (11%). However, our study demonstrated that the majority of patients had normal radiological findings, while a minority demonstrated pneumonic shadow with no typical findings or correlations with severity. Similar results have been reported in a couple of studies (7, 25).

Typical abnormal laboratory findings did not show significant differences among different cohorts. Xia et al. (31) reported laboratory findings in pediatric patients with COVID-19, including lymphopenia (35%) and elevated ALT (25%), creatine kinase-MA (75%), CRP (45%), and procalcitonin (80%) levels. The main laboratory findings found in our study were lymphopenia (18.8%) and elevated CRP (71.88%), D-dimer (91.67%), ALT (6.2%), and AST (6.25%) levels. A study from SA reported lymphopenia and high D-dimer and CRP levels in most cases (25). Another retrospective study conducted in SA showed that patients who were admitted to the PICU had higher laboratory ALT, AST, and creatinine levels at admission than those who were not admitted to the PICU (6). Further, a study of adult patients with COVID-19 demonstrated that prothrombin time and D-dimer levels were higher in patients admitted to the ICU than those who were not (34). Zhou et al. (35) reported that an elevated D-dimer level was one of the important risk factors of death in adult patients after COVID-19. Our study showed high D-dimer levels in all PICU-admitted cases (100%).

Despite the fact that most children with COVID-19 have a mild course, some develop an extensive systemic inflammatory response known as MISC, which is similar to KD and can affect numerous organs, including the gastrointestinal system, skin, lymph nodes, and others; it can manifest with shock, cardiac dysfunction, acute heart failure, and extremely high levels of inflammatory biomarkers and the brain natriuretic peptide (36–38). Almoosa et al. (39) reported 10 cases of MIS-C in the eastern area of SA with wide-spectrum diseases and eventually with good outcomes. Only four patients in our cohort with MISC-associated COVID-19 developed a severe inflammatory phenotype, including fever, gastrointestinal symptoms, shock, cardiac dysfunction, liver and kidney dysfunction, and coagulopathy, as well as clinical features that overlapped with KD, such as rash, conjunctivitis, swelling of the extremities, and oropharyngeal redness; all patients recovered without sequelae and responded well to usual treatments, such as immunoglobulin or steroid therapy.

Protocols have changed periodically as evidence has arisen regarding the treatment of COVID-19. Many trials were conducted during the beginning of the pandemic, and at the time of writing this paper, no Food Drug Administration-approved drugs for the treatment of COVID-19 have been released. In our study, most of the cases were severe and pediatric patients recovered with oxygen (71.88%) as an interventional treatment followed by antibacterial antibiotics (48.96%).

The implication for practice of this study will enlighten and increase the knowledge of the health care providers about the disease unique characteristics and their outcomes to detect it early in order to deliver the optimum management specially for the critically ill patients. Also, awareness campaigns for the public on keeping all preventive measures, such as wearing face masks, social distancing, and complying with good hand hygiene practice, will prevent the spread of the infection. Additionally, the importance of vaccination in pediatric age groups is crucial.

CONCLUSIONS

In conclusion, our findings showed that the majority of the admitted children were $\leq$3 years of age (52.1%) and infected with an unknown source (56.2%). Moreover, the majority of the cases had severe COVID-19 infection as they required oxygen (71.88%). In addition, the patients presenting with respiratory symptoms and fever were associated with high CRP and D-dimer levels, but most of them had a favorable outcome (97.92%). However, some cases were critical and resulted in death. Early detection of COVID-19's unique features in pediatric patients and quick treatment are vital for critically ill patients. Due to the continuous spread of the virus and increasing infection rate, future studies will be critical to better understanding the disease spectrum and potential therapeutic options.

Limitations

The results represent an early stage of COVID-19 infection and transmission at one site, and we only had a small number of critical cases, which is one of the study's major limitations. In addition, this study was done during the first wave of the pandemic and that other variants of COVID-19 have appeared since then. Moreover, a number of significant elements changed over this time, including the implementation of social distancing, national lock-down measures, and increased testing speed, all of which could have altered the true incidence and outcome data. In addition, revisions in the SMOH's recommendations might have affected the results. Lastly, as a retrospective cohort study, it was difficult to maintain a long follow-up period, so the study is susceptible to some loss of data—we excluded five patients with incomplete data. Moreover, the lack of a control group (not exposed) to compare with the exposed group so the relative risk of the exposure can be calculated.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by King Fahad Medical City. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AA: conceptualization, investigation, writing—original draft, writing—review and editing, visualization, and project administration. DA: conceptualization, investigation, writing—review and editing, visualization, and project administration. SAlG, SAlF, and EA: investigation, methodology, and writing—review and editing. YB: formal analysis, data curation, and writing—review and editing. TA and LA-A: methodology, investigation, and writing—review and editing. MA: methodology and investigation. YA: formal analysis, resources, writing—original draft, writing—review and editing, supervision, and project administration. All authors contributed to the article and approved the submitted version.

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Comparison of Respiratory Pathogens in Children With Lower Respiratory Tract Infections Before and During the COVID-19 Pandemic in Shanghai, China

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Objectives: This study aimed to assess the impact of COVID-19 on the prevalence of respiratory pathogens among hospitalized children with lower respiratory tract infections (LRTIs) in Shanghai.

Methods: Respiratory specimens were collected from children with LRTIs in Children's Hospital of Fudan University from February 2019 to January 2021 and common respiratory pathogens were detected using multiplex PCR. The data of 13 respiratory pathogens were analyzed and compared between the year of 2020 (from February 2020 to January 2021) and 2019 (from February 2019 to January 2020).

Results: A total of 1,049 patients were enrolled, including 417 patients in 2019 and 632 patients in 2020. In 2020, 27.53% of patients were tested positive for at least one pathogen, which was significantly lower than that in 2019 (78.66%). The top three pathogens were Mycoplasma pneumoniae (Mp), human adenovirus (ADV) and human rhinovirus (RV) in 2019, whereas RV, human respiratory syncytial virus (RSV) and human parainfluenza virus (PIV) were the predominant ones in 2020. The positive rates of Mp, ADV, RV, PIV, Influenza virus B (InfB), H3N2, and H1N1 were significantly decreased in 2020. RV was the most detectable respiratory pathogen in 2020, and become the most frequent pathogen in all five age groups. PIV had a high prevalence from October to December 2020 which was even higher than that in 2019. Influenza virus A (InfA) was not detected in 2020. Co-infection was significantly less frequent in 2020.

Conclusion: The public health interventions aiming to eliminate COVID-19 have great impact on the prevalence of common respiratory pathogens. The prevalence of RV and PIV reminds us a possible resurgence of some pathogens.

Keywords: COVID-19, respiratory pathogens, prevalence, children, lower respiratory tract infections

INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan China, in December 2019 (1). In Shanghai, the first COVID-19 patient was identified on 20 January 2020. For the substantial growing outbreak, the World Health Organization (WHO) declared a pandemic on 11 March 2020 (2).

Soon after the emergence of community transmission of COVID-19, the Chinese government has introduced progressive border restrictions. The following nationwide non-pharmaceutical interventions (NPIs) were ordered aiming to eliminate COVID-19, such as prohibiting social gatherings, wearing masks, hand hygiene, postponing the spring 2020 semester in primary and middle schools, and even canceling all face-to-face teaching in schools (3). Remote work at home was also recommended for employed people whenever possible. These evidence consisted of global experience and previous pandemics (4). In previous studies, social distancing could reduce the spreading of seasonal influenza in workplaces (5). The real effect of these NPIs on the spreading of diseases remains unknown, but it appeared to effectively eliminate community transmission of COVID-19 in China in 2020 (6). Studies from Australia, New Zealand, Japan, and the United States also observed the decrease of respiratory viruses, especially seasonal influenza and human respiratory syncytial virus (RSV), when compared with previous seasons (7–10).

Lower respiratory tract infections (LRTIs) are one of the most common childhood diseases and a leading cause of morbidity and mortality in children especially those under 5 years old (11). A number of pathogens are capable of causing LRTIs including bacteria, virus, fungi and atypical pathogen, among which viral respiratory tract infection is the leading cause (appropriate 80%) (12). The major viral pathogens include influenza virus, RSV, coronavirus, adenovirus, and rhinovirus. Previous study showed that in children below 5 years, the combined global mortality of only influenza and RSV reaches 300 000 deaths each year (13). *Mycoplasma pneumoniae* (Mp) is also considered a common cause of community-acquired pneumonia. Mp is identified to be responsible for up to 40% of community-acquired pneumonia in children elder than 5 years (14, 15).

However, there were few studies of how the respiratory pathogens and clinical epidemiological features changed during the outbreak of COVID-19 in Shanghai. To evaluate the prevalence of common respiratory pathogens among hospitalized children with LRTIs in Shanghai in 2020, and to compare it with the prevalence patterns in the year of 2019, we explored the changes in epidemiology of the common respiratory pathogens from February 2019 to January 2021.

MATERIALS AND METHODS

Study Subjects and Ethics Statement

In this study, respiratory specimens (nasopharyngeal aspirates/bronchoalveolar lavage fluid) were obtained from 1,049 enrolled children who were treated for LRTIs in Children's Hospital of Fudan University by trained staff following standard operating procedures, between February 2019 and January 2021. The specimens were immediately transferred to the clinical laboratory for respiratory pathogens detection. The inclusion criteria were (1) children aged younger than 18 years; (2) hospitalization at the Children's Hospital of Fudan University; (3) diagnosed with LRTIs (presenting with at least one of the following signs/symptoms: fever, cough, sputum, shortness of breath, lung auscultation abnormality (rale or wheeze), tachypnea, and chest pain (16). Demographics and clinical data from enrolled children were obtained from their electronic medical records. The project was approved by the Ethical Committee of Children's Hospital of Fudan University.

The patients were divided into five age groups: under 28 days of age, 1–12 months of age, 1–3 years of age, 4–6 years of age, and more than 7 years of age.

Specimen Detection

A SureX® 13 Respiratory Pathogen Multiplex Kit (Health Gene Technologies, Ningbo, China) was used to detect respiratory pathogens in accordance with the recommended protocol including general influenza virus A (InfA), influenza virus A H1N1 (2009) (H1N1), the seasonal influenza virus A H3N2 (H3N2), influenza virus B (InfB), human adenovirus (ADV), human Boca virus (Boca), human rhinovirus (RV), human parainfluenza virus (PIV), human metapneumovirus (MPV), human coronavirus (CoV), human respiratory syncytial virus (RSV), Chlamydia (Ch), and Mycoplasma pneumoniae (Mp) in all the collected specimens.

Statistical Analysis

Statistical analyses were conducted using SPSS 23.0 software (IBM, New York, United States). Categorical variables were expressed as numbers (%). Continuous variables were expressed as medians (interquartile range). Proportions for categorical variables (detection rates of virus, sex, and age) were compared using the chi-square test. All of the tests were two-tailed, and a value of $P < 0.05$ represented statistical significance.

RESULTS

During the study period, 1,049 patients who met the inclusion criteria were enrolled, including 417 patients in 2019 (from February 2019 to January 2020) and 632 patients in 2020 (from February 2020 to January 2021). The most common clinical diagnoses were pneumonia, bronchitis and bronchiolitis. The intensive care unit admission rate was 9.11% (38/417) and 5.85% (37/632) in the year of 2019 and 2020, respectively. Among all the enrolled patients, 12 deaths in hospital in 2019 and 8 deaths in hospital in 2020 occurred. No significant difference was observed in sex ratio between the year of 2019 and 2020. The median age of patients in 2020 (2.8 months) was much less than that in 2019 (21.5 months) (**Table 1**). To be specific, the proportion of children aged 0–28 days (220/632, 34.81% vs. 46/417, 11.03%, $P = 0.000$) and 1–12 months (230/632, 36.39% vs.

TABLE 1 | Characteristics and incidence of detected respiratory pathogens in hospitalized patients with lower respiratory tract infections (LRTIs) during the year of 2020 (February 2020 to January 2021) compare with the year of 2019 (February 2019 to January 2020).

	2019 (*n* = 417)	2020 (*n* = 632)	*P*-value
Characteristics			
Age, median (IQR), m	21.5 (4.4–60.0)	2.8 (0.5–16.2)	0.000
Male sex, n (%)	232 (55.64)	384 (60.76)	0.109
Pathogens			
Mp	119 (28.54)	16 (2.53)	0.000
ADV	89 (21.34)	8 (1.27)	0.000
RV	88 (21.10)	82 (12.97)	0.001
PIV	32 (7.67)	23 (3.64)	0.007
RSV	25 (6.00)	25 (3.96)	0.140
InfB	17 (4.08)	1 (0.16)	0.000
H3N2	16 (3.84)	0 (0.00)	0.000
MPV	10 (2.40)	9 (1.42)	0.249
H1N1	6 (1.44)	0 (0.00)	0.004
Ch	6 (1.44)	10 (1.58)	1.000
Boca	5 (1.20)	3 (0.47)	0.277
CoV	4 (0.96)	8 (1.27)	0.772
InfA	1 (0.24)	0 (0.00)	0.398
Co-infection	80 (19.18)	11 (1.74)	0.000
Total	328 (78.66)	174 (27.53)	0.000

115/417, 27.58%, *P = 0.003*) in 2020 increased significantly when compared with that in 2019.

In 2020, of 632 patients, 174 (27.53%) tested positive for at least one of the 13 pathogens, which was significantly lower than that in 2019 (328/417, 78.66%) (*P = 0.000*) (**Table 1**). The top three pathogens were Mp, ADV and RV in 2019, whereas RV, RSV and PIV were the predominant ones in 2020. The positive rates of Mp, ADV, RV, PIV, InfB, H3N2, and H1N1 were significantly decreased in 2020, when compared with that of 2019. Other pathogens, like RSV, MPV and Boca presented a lower positive rate in 2020 with no statistical difference. InfA, including HIN1 and H3N2, was not detected in 2020. The most common respiratory pathogens that caused intensive care unit admission were ADV (39.47%, 15/38), RV (18.42%, 7/38), PIV (7.89%, 3/38), and Mp (7.89%, 3/38) in the year of 2019, whereas RV (13.51%, 5/37), ADV (5.41%, 2/37), and PIV (5.41%, 2/37) were the predominant ones in the year of 2020. In the death patients in 2019, the most associated pathogens were ADV (41.67%, 5/12), PIV (8.33%, 1/12), and RV (8.33%, 1/12). RSV (12.50%, 1/8) was the only pathogen found in the death patients in 2020.

Compared with 2019, dramatic reductions were observed for positive rates of the respiratory pathogens after February 2020 (WHO declared public health emergency of international concern) and kept at a lower positive rate (**Figure 1**). RV was the only respiratory pathogen that remained seasonal distribution pattern with a higher prevalence in June and September. In addition, the positive rate of RV in September 2020 was much lower than that in 2019 (21.82 vs. 54.29%) (**Figure 2A**). RSV outbreaks occurred in December 2019, but the detection rates in the other months were very low. The detection peak of RSV in 2020 was observed in January 2021 (**Figure 2B**). PIV had a high prevalence from March to August (spring and summer) in 2019, while it was almost not detected in the same period in 2020. Instead, a high prevalence of PIV was observed from October to December 2020 which was even higher than that in 2019 (**Figure 2C**). The positive rate of MPV peaked in February in 2019 and in January in 2021, respectively (**Figure 2D**). Mp was rarely detected in 2020 with a total positive rate of 2.53%. Mp has a clear seasonal distribution pattern as it peaked in July in 2019, and the positive rate of Mp is significantly higher than that in each month of 2020 except February (**Figure 2E**). We observed that ADV was detected throughout 2019 with a higher prevalence in the first half-year, whereas the phenomenon was not observed in 2020. Instead, ADV is distributed almost evenly throughout the year except for a slight increase in the positive rate in February 2020 (**Figure 2F**).

The detection rates for respiratory pathogens in all five age groups in 2020 were much lower than that in 2019. The predominant pathogens among different age groups varied between 2019 and 2020. In 2019, for children aged 0–28 days, RSV accounted for 31.82% of pathogens that caused LRTIs, and Mp was not detected. The proportion of RSV decreased with age, and the proportion of Mp increased with age, yet Mp was the most frequent respiratory pathogen in children over 4 years. RV and ADV occupied the majority in 1–12 months and 1–3 years age group in 2019. However, the proportion of RV increased in every age group, and appeared to be the most common respiratory pathogen in all five age groups in 2020 (**Figure 3**).

Co-infection was still observed in 2020 (11/632, 1.74%), but with significantly less frequency than that in 2019 (80/417, 19.18%). RV was the most frequent pathogen in co-infection in 2020, while Mp ranked the first in 2019 of all mixed infections. Ten triple-infections were found in 2019, whereas no triple-infections was detected in 2020 (**Table 2**).

DISCUSSION

Studies from many countries have yielded that outpatient and inpatient visits associated with diagnosed infections fell markedly during the COVID-19 pandemic (17–20). These infectious diseases, including LRTIs, are predominantly transmitted through droplets, aerosols, or physical contact which were also believed to be the major transmission routes of COVID-19. In this study, we retrospectively analyzed the prevalence of the most common respiratory pathogens among hospitalized children with LRTIs before and during COVID-19 pandemic. Our study showed that the overall detection rates of respiratory pathogens were 78.66% in 2019 and 27.53% in 2020. It seemed that the series of strict NPIs adopted to contain the COVID-19 pandemic, including wearing masks, hand hygiene, closing schools and social distancing had led to a greatly reduced transmission of LRTIs and affected the prevalence of the common respiratory pathogens.

Interestingly, the proportion of children under 1 year in the number of specimens increased significantly in our study in 2020, thus the median age in 2020 was much less than that in 2019.

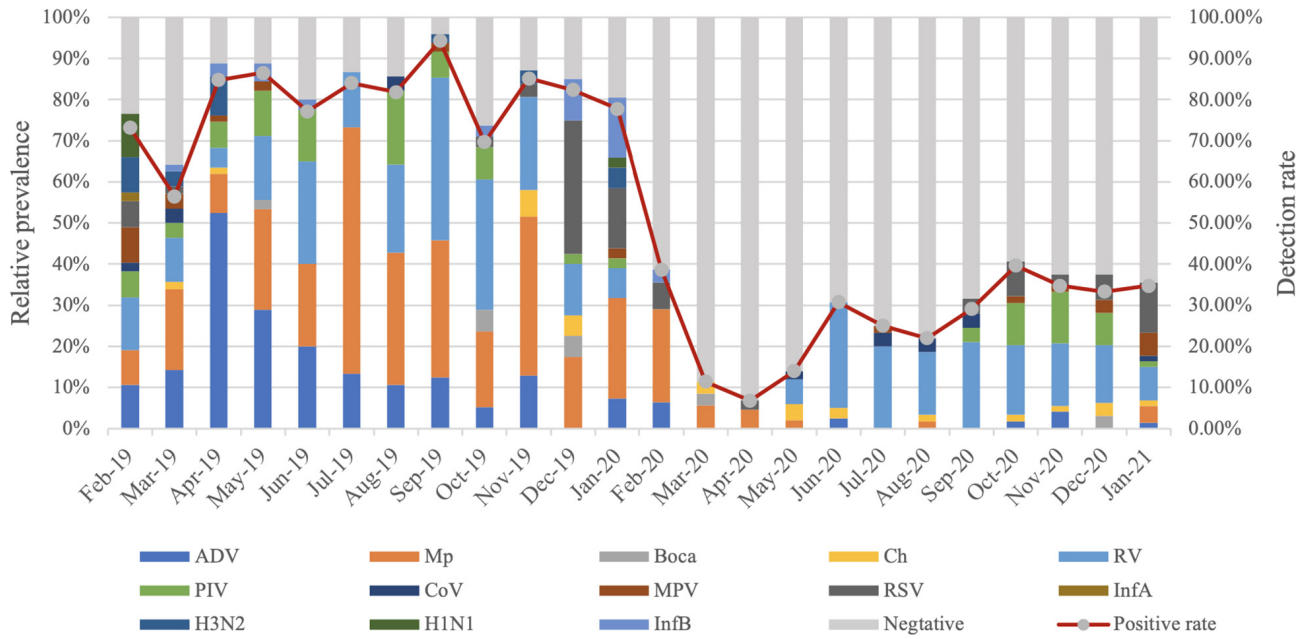

FIGURE 1 | Month-wise distribution of respiratory pathogens detected in hospitalized patients with lower respiratory tract infections (LRTIs) from February 2019 to January 2021.

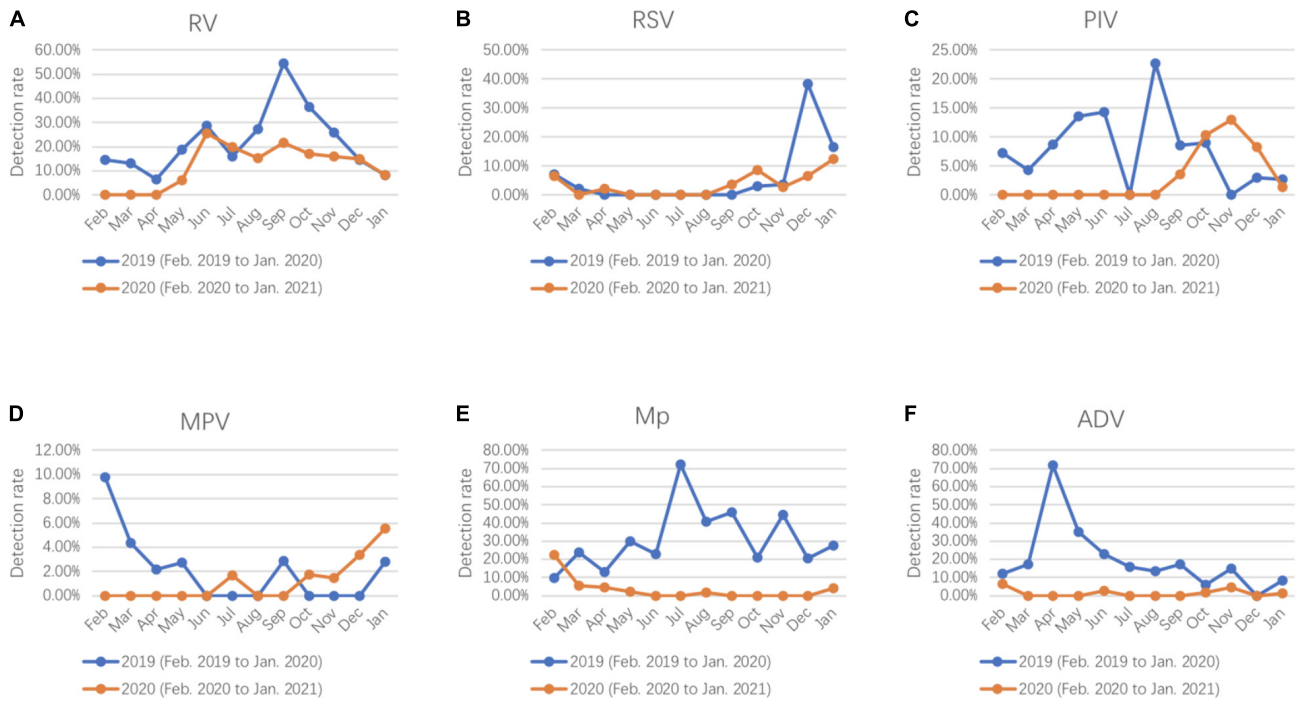

FIGURE 2 | Seasonal activity of respiratory pathogens **(A)** RV, **(B)** RSV, **(C)** PIV, **(D)** MPV, **(E)** Mp, and **(F)** ADV detected in hospitalized patients with lower respiratory tract infections (LRTIs) during the year of 2020 (February 2020 to January 2021) compare with the year of 2019 (February 2019 to January 2020).

One possible reason is that the NPIs adopted in the pandemic like closing schools and social distance reduced the outdoor activities of the elder children to a more significant extent. Meanwhile, younger children had poor compliance for the NPIs, as the use of masks, hand washing and the use of gel alcohol before and after contact with other people. Furthermore, for a long time at the

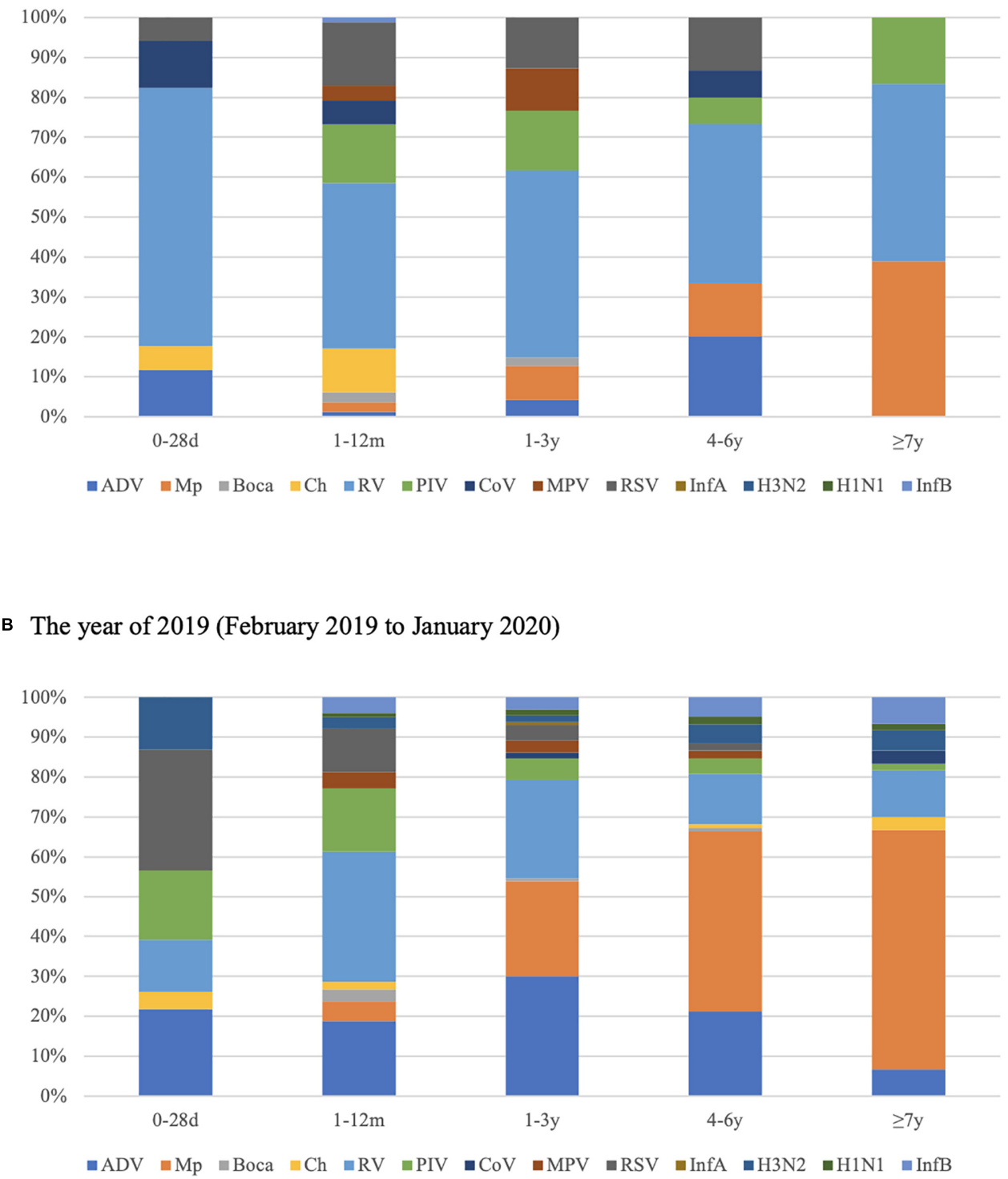

FIGURE 3 | Proportions of respiratory pathogens detected in 2020 (February 2020 to January 2021) **(A)** and 2019 (February 2019 to January 2020) **(B)** according to age group.

beginning of the pandemic, less suitable masks were available for children under 1 year, which also made younger children more vulnerable to respiratory pathogens.

It is known to all, the WHO's pandemic influenza intervention guidance does not recommend stringent NPIs when pandemic influenza reaches sustained transmission in the population (21). In this study, we have observed an almost absence of the usual winter influenza virus epidemic with a remarkable reduction of InfA, H1N1, H3N2, and InfB during the COVID-19 pandemic, which was also observed in many other areas, including Hong Kong, New Zealand, Finland, Australia, and France (7, 8, 22–24). A previous study found that surgical face masks significantly reduced the detection of influenza virus RNA in respiratory droplets, indicating wearing masks could prevent the transmission of influenza viruses from individuals (25). These results demonstrated that the NPIs, including international mobility restriction, hands-washing, especially masks-wearing could highly reduce the spread of influenza.

In our study, RV was the most detectable respiratory pathogen in 2020, and become the most frequent pathogen in all age groups. Before the re-opening of school (early June, 2020), an absolutely low detection rate of RV was observed. However, after June 2020, there was a sharp increase in the number of detections. Previous studies in Austria, New Zealand, and Japan have also reported a high incidence of RV infection during COVID-19 pandemic (7, 26–28). However to the best of our knowledge, the mechanism that accounts for this phenomenon remains unclear. One possible reason is that RV is a non-enveloped virus, which is relatively resistant to ethanol-containing disinfectant (29) and it can survive on the environment surfaces for a prolonged period of time (30). Additionally, RV infections, are frequently transmitted within households from children to other family members (31). These factors might have contributed to RV infection being less affected by the NPIs.

Remarkably, the prevalence of PIV was basically undetectable from January to August 2020, and an obvious increase in the detections of PIV was observed in the winter season, which were even higher than the same period in 2019. Previous studies showed that PIV plays an important role in hospitalized children with LRTIs with a peak detection in spring and summer, which was similar to the seasonality in 2019 (32). Unlike the previous report, the seasonality of PIV circulation has changed in 2020. The peak detection rate was observed in November 2020. The resurgence was also observed on MPV after October 2020. A study in Shenzhen also observed that the detection rates of PIV and MPV increased from September to December 2020 (33). Except the NPIs, other factors, such as virus competition, the immune response or interference through viral proteins, may also contribute as well. Previous studies have reported that viral interference among influenza virus, rhinovirus, and other respiratory viruses can affect viral infections at the host and population level (34, 35). The lower detection rate of other respiratory pathogens, especially the absence of influenza virus due to the NPIs may partially explain the rise in PIV and MPV. Therefore, we speculate that PIV and MPV should be monitored more diligently in children in the future.

It is reported that the seasonality of RSV has significant changed and a sharp increase in RSV detections occurred at the end of the winter season in Australian in 2020 (26). Another report from New Zealand showed no increase in RSV detections during the whole 2020 winter season (7). Unlike these studies, the detection rate of RSV in our study was much lower in 2020, and the peak detection rate was observed later in January 2021. RSV mostly spreads among the youngest children, however, with the higher proportion of children under 1 year in our study, no higher detection rate of RSV was observed. The mechanism behind this finding is unclear, continued monitoring of RSV epidemic trends and further research are needed.

Like most respiratory pathogens, Mp infection usually occurs during winter months but can happen year-round, that was consistent with the seasonal pattern of Mp in 2019. It is notable that the prevalence of Mp was markedly decreased in 2020 and showed no obvious seasonality. Meanwhile, the detection rate of Mp decreased sharply in all five age groups and the detection rates of Mp in 4–6 years and $\geq$7 years age groups were as high as 57.31% (47/82) and 59.02% (36/61) in the year of 2019 while they decreased to 5.41% (2/37) and 13.73% (7/51) in the year of 2020. For Mp is transmitted from person to person via respiratory droplets during close contact, outbreaks of Mp infection often occur in military recruits, hospitals, nursing homes, and other long-term care facilities (14, 15). In addition to the NPIs that have been implemented, the chances of children, who are the main reservoirs of Mp, spreading disease in the community may be reduced because they have not been attending childcare facilities and schools during the pandemic. This might partly explained the disappearance of Mp.

An obvious detection peak of ADV was observed in the spring of 2019, which indicated an ADV outbreak in 2019. This was consistent with the results reported by Kong et al., that a small explosive epidemic of adenovirus occurred with a high positive rate in Shanghai from January to May in 2019 (36). Adenovirus-associated severe pneumonia was the leading clinical diagnosis of death in hospital in 2019 which may be related to the ADV outbreak in 2019. However, no detection peak was observed in 2020 with low incidence throughout the year. Because of no consistent seasonal pattern of ADV, further investigation is required to establish the association.

TABLE 2 | Co-infections of respiratory pathogens in hospitalized patients with lower respiratory tract infections (LRTIs) during the year of 2020 (February 2020 to January 2021) compare with the year of 2019 (February 2019 to January 2020).

	2019 (*n* = 417)	2020 (*n* = 632)
Double infections, n (%)		
ADV + Mp	15 (3.60)	0 (0.00)
RV + Mp	12 (2.88)	0 (0.00)
ADV + RV	9 (2.16)	0 (0.00)
ADV + PIV	6 (1.44)	2 (0.32)
RV + PIV	4 (0.96)	3 (0.47)
Others	24 (5.76)	6 (0.95)
Triple infections, n (%)		
ADV + RV + Mp	4 (0.96)	0 (0.00)
Others	6 (1.44)	0 (0.00)

Our study is limited in that all of the data were obtained from one hospital to introduce selection bias. Furthermore, the changes in bacteria were not observed before and during the COVID-19 outbreak. Continued monitoring of viral epidemic trends and further research are needed to help us to better control the epidemic outbreak and correctly handle LRTIs.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of Children's Hospital of Fudan University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MX and JX conceived and designed the experiments and wrote the manuscript. MX, PL, HZ, and LL collected the respiratory samples. MX, LS, and LC performed the experiments and collected the clinical data. MX, PL, LL, and RJ analyzed the data. All authors contributed to the article and approved the submitted version.

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Correlation of SARS-CoV-2 Viral Load and Clinical Evolution of Pediatric Patients in a General Hospital From Buenos Aires, Argentina

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Background: SARS-CoV-2 infection is associated with a wide range of clinical manifestations and severity. Pediatric cases represent <10% of total cases, with a mortality rate below 1%. Data of correlation between SARS-CoV-2 viral load in respiratory samples and severity of disease in pediatric patients is scarce. The cycle threshold (CT) value for the detection of SARS-CoV-2 could be used as an indirect indicator of viral load in analyzed respiratory samples.

Objective: The aim of this study was to describe CT values and their correlation with clinical manifestations, epidemiology and laboratory parameters in pediatric patients with confirmed COVID-19.

Methods: In this observational, retrospective, analytic and single-center study we included patients under 15 years with confirmed COVID-19 by RT-PCR SARS-CoV-2 admitted to the Isidoro Iriarte Hospital (Argentina) between March 1st 2020 and April 30th 2021.

Results: 485 patients were included, the distribution according to disease severity was: 84% (408 patients) presented mild disease, 12% (59 patients) moderate disease and 4% (18 patients) severe disease. Patients with moderate and severe illness had an increased hospitalization rate, prolonged hospitalization, higher frequency of comorbidities and oxygen and antibiotics use. CT values, that could be used as an indirect measure of viral load, was associated with severity of clinical manifestations and age under 12 months. No patient required admission to PICU nor mechanical ventilation. No deaths were registered.

Conclusions: In this study, the viral load of SARS-CoV-2 in respiratory samples, determined by the cycle threshold, was significantly correlated with moderate to severe cases and with age.

Keywords: COVID-19, SARS-CoV-2, children, viral load, cycle threshold (CT) value

INTRODUCTION

Cases of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were first reported in late-December 2019. In March 2020 the World Health Organization (WHO) declared the COVID-19 outbreak as a pandemic. By August 12th 2021, 200 million cases and more than 4 million deaths were reported worldwide and 5 million cases and more than 100 thousand deaths in Argentina (1). In Argentina, 10% of the cases affecting people under 19 years of age with a fatality rate of 0.05% mainly associated with comorbidities and age (age group under 12 months) (2).

In the pediatric population COVID-19 severity was associated with the presence of certain comorbidities such as cancer, diabetes mellitus, neurological diseases, among others (3, 4).

The CT (cycle threshold) values in a RT-PCR (reverse transcription real time polymerase chain reaction) assay is defined as the number of cycles required for the fluorescent signal to cross a certain threshold. CT levels are inversely proportional to the amount of target nucleic acid in the sample and can be an indirect measure of viral load of the samples. Although there is no direct viral load quantification, it can be extrapolated that each 3.3 increase in CT value correlates with ∼1 log (i.e., 10-fold) less target in the nasopharyngeal sample undergoing the PCR. In line with this idea, it is possible to determine CT ranges of the different samples as indicators of high (CT below <25), moderate (CT between 25 and 35) and low (CT over 35) viral loads.

Since COVID-19 outbreak, several reports have tried to address whether viral load and illness severity are correlated in adult and pediatric patients. The aim of this study is to contribute with this topic focusing on the correlation of SARS-CoV-2 viral load in respiratory samples with clinical severity. Additionally, epidemiological, clinical characteristics and laboratory parameters of COVID-19 positive patients are analyzed.

METHODS

Study Design

An observational, retrospective and analytic study was conducted including patients aged <15 years with a RT-PCR SARS-CoV-2 test positive who attended to the Hospital Zonal General de Agudos "Dr. Isidoro G Iriarte" from Quilmes, Buenos Aires, Argentina between March 1st, 2021 and April 30th, 2021.

Inclusion Criteria

Patients with confirmed SARS-CoV-2 infection by RT-PCR in samples from the upper respiratory tract.

Exclusion Criteria

Clinical or epidemiological COVID-19 diagnosis, diagnosis by immunochromatographic methods or patients with incomplete clinical data.

Definition

Severity of COVID-19 was defined by clinical and complementary evaluation according to Dong et al. (5).

(1) Asymptomatic infection: no symptoms nor clinical signs, normal chest X-ray, positive SARS-CoV-2 RT-PCR; (2) mild: acute infection symptoms in the upper respiratory tract, including fever, fatigue, myalgia, cough, odynophagia, rhinorrhea, normal auscultation. Some cases presented no fever or had only digestive symptoms such as abdominal pain, diarrhea, nausea, and vomiting; (3) moderate: Pneumonia, persistent fever and cough-mainly dry cough followed by productive cough-, wheezing without dyspnea nor hypoxemia. Asymptomatic cases with subclinical pulmonary lesions determined by chest CT scan; (4) severe: early respiratory symptoms, fever and cough, presented with gastrointestinal symptoms such as diarrhea. Disease progression observed within 1 week reaching dyspnea with central cyanosis. Oxygen saturation of ≤92% with other hypoxia manifestations; (5) or critical: rapid progression to acute respiratory distress syndrome or respiratory insufficiency, shock, encephalopathy, myocardial lesion or cardiac insufficiency, coagulation disorders and acute renal lesions.

Detection of SARS-CoV-2 RNA from samples from the upper respiratory tract was performed by RT-PCR. Nasopharyngeal swabs (combined swab) or nasopharyngeal aspirates (patients under 1 year) were collected following institutional and national recommendations. Samples were analyzed in the COVID-19 Unit of the Universidad Nacional de Quilmes (UNQ). Different commercial kits were used: GeneFinder™ COVID-19 Plus RealAmp kit (OSANG Healthcare, Korea) and Quantabio Script One-Step RT-PCR (Genbiotech SRL) for RNasa P detection; Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 (BGI, China); DisCoVery SARS-CoV-2 RT-PCR detection kit Rox o Cy5 (AP-Biotech, Argentina); COVID-19 One-Step RT-PCR Kit FAPON (FAPON Biotech Inc, China). Viral load on respiratory samples was estimated using cycle threshold (CT): high viral load (CT < 25), intermediate viral load (CT 25–35) and low viral load (CT > 35). The samples were analyzed according recommendations of manufacturer.

Different kits were used to detect SARS-CoV-2 RNA due availability of resources in the laboratory and the province of Buenos Aires. All of these commercially available tests were approved by the Ministry of Health of the Province of Buenos Aires and the Administración Nacional de Medicamentos, Alimentos y Tecnología Sanitaria (ANMAT). All of these kits are proven to have high efficiency. Given the differences found in cut-off values among the different commercial kits, in this work we analyzed ranges of CT values to circumvent this issue. Three different CT ranges were used <25, 25–35, and >35, to address high, moderate or low viral loads.

Statistical Analysis

Continuous variables were analyzed using the *t*-test or the Wilcoxon test for those with parametric normally distributed or non-parametric distributed data, and were expressed as a mean and standard deviation or median and interquartile range, respectively. For categorical variables Chi-square test with Yates or Fisher correction was used and these were expressed as percentages. Univariate analysis was performed to identify potential predictors of severity and a multiple logistic regression

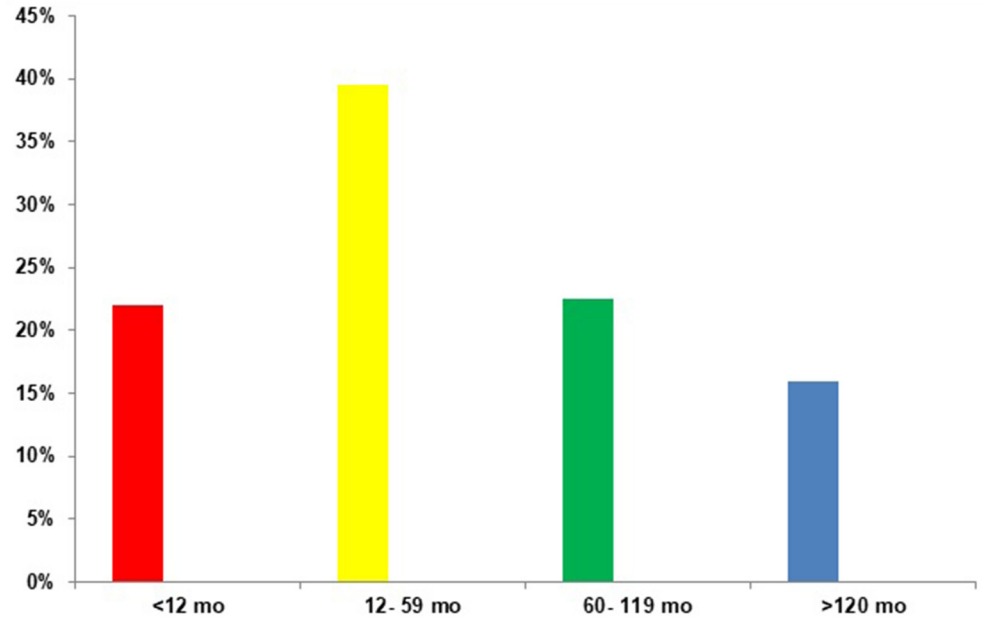

FIGURE 1 | Distribution of the included population according to age ($n = 485$).

model was performed to identify predictive variables for severe infection and COVID-19 fatality in the pediatric population analyzed. Variables with significant association in the initial analysis ($p < 0.2$) and/or those considered to be clinically relevant were analyzed using Wald test. To this realized model, we sequentially added age and comorbidities and other clinical or analytical parameters that were significant in the univariate analysis. All the variables were analyzed individually to find an independent relation with outcomes. A value of $p < 0.05$ was considered significant.

Ethics

This study was approved for the Research Committee Board of the Hospital Isidoro Iriarte Number 65/2020. The information was processed following the guidelines of the Helsinki Declaration, the CIOMS guidelines and the national laws that determine the handling of people's data. The signing of an informed consent was not required as it was considered a low-risk investigation, with no interventions outside of standard care.

RESULTS

The study population included 485 patients (p), 53% (257 p) were male infants with a median age of 36 months. The distribution according to age is shown in **Figure 1**. All cases were community transmitted, identifying household/family transmission in 23% of them (110 p). All patients were symptomatic, being the most common symptoms at the onset of illness fever [398 p, (82%)], cough [252 p, (52%)], vomiting [145 p, (30%)], diarrhea [116 p, (24%)], dyspnea [87 p, (18%)], odynophagia [82 p, (17%)], abdominal pain [78 p, (16%)] and headache [63 p, (13%)]. According to the clinical severity, 84% (408 p) of the cases were mild, 12% (59 p) moderate and 4% (18 p) severe. No critical patients were observed. Respiratory symptoms were associated with moderate and severe cases ($p < 0.01$), while gastrointestinal symptoms were more frequent in mild cases ($p < 0.001$). Interestingly, 21% (103 p) of patients had underlying comorbidities and these patients presented moderate and severe cases ($p < 0.001$).

Of the 485 analyzed patients, 20% (97 p) required hospital admission and patients with moderate and severe illness had more frequent and prolonged hospitalization ($p < 0.05$). No significant differences were observed in hospital admission rates for all age groups. From hospitalized patients, 10% (49 p) required oxygen therapy and 6% (28 p) received antimicrobial therapy, being more frequent in severe cases ($p < 0.05$).

Interestingly, patients with moderate and severe illness showed a higher viral load ($p < 0.001$). In this regard, the viral load was higher in those under 12 months of age.

No patients required to be transferred to the pediatric intensive care unit (PICU) and no patient died. The variables associated with clinical and age stratification are shown in **Tables 1**, **2**, respectively, and the distribution of symptoms according viral load (CT-PCR-SARS-CoV-2) is shown in **Figure 2**.

DISCUSSION

In this study, 96% of patients had mild and moderate symptoms. These results are in line with most pediatric reports, showing mainly asymptomatic cases or mild to moderate disease.

TABLE 1 | Presenting characteristics of the analyzed population.

Variable	Mild ($n = 408$)	Moderate ($n = 59$)	Severe ($n = 18$)	*p*
Males (*n*, %)	212 (52 %)	37 (63%)	8 (44%)	NS
Age in months (median, IQR)	36 (17–108)	36 (14–108)	42 (16–87)	NS
Comorbidities (*n*, %)	65 (16%)	31 (53%)	7 (39%)	<0.001
Days of evolution (median, IQR)	4 (3–5)	1(1–2)	2 (1–3)	NS
Hospitalization (*n*, %)	23 (5%)	56 (95%)	18 (100%)	<0.05
Hospitalization days (median, IQR)	3 (2.3–3.7)	4 (3–5)	5 (3–7)	<0.05
Supplementary oxygenation (*n*, %)	5 (1%)	29 (49%)	15 (83%)	<0.001
Days of supplementary oxygenation (median, IQR)	2 (0.9–3.1)	2 (1.7–2.3)	2 (1.56–2.44)	NS
Antibiotic treatment (*n*, %)	5 (1%)	12 (20%)	11 (61%)	<0.05
Days of antibiotic treatment (median, IQR)	5 (1.3–8.7)	5 (2.7–7.3)	5 (3.5–6.5)	NS
Cycle threshold (*n*, %)				<0.001
<25	58 (14%)	19 (32%)	12 (67%)	
25–35	278 (68%)	35 (59%)	6 (33%)	
>35	72 (18%)	5 (9%)	0 (0%)	
Fever (*n*, %)	357 (87.5)	33 (57)	10 (56)	< 0.001
Cough (*n*, %)	198 (48.5)	40 (66)	14 (78)	<0.01
Odynophagia (*n*, %)	79 (19.4)	4 (7)	0 (0)	<0.05
Respiratory distress (*n*, %)	48 (11.8)	27 (45.8)	13 (72)	< 0.001
Diarrhea (*n*, %)	111 (27.7)	3 (5)	1 (5.6)	< 0.001
Vomiting (*n*, %)	140 (35)	6 (10)	1 (5.6)	< 0.001

Distribution according to disease severity (n = 485).

Regarding the classification of clinical severity, although we follow the one suggested by Dong et al., we consider that it would be necessary to reevaluate its application in clinical practice, as suggested by Buonsenso et al. (6) in their letter to the author. In our center, tomographic images were not performed on patients without clinical manifestations, so the parameter of pathological findings on chest tomography in asymptomatic patients was not used to classify patients as moderate (4–8).

There were no differences in the proportion of male and female patients (53% of patients were male) and the clinical outcomes were not associated with sex. This differs from other reports in adults that showed that clinical infection is more likely to be more severe with higher mortality rates in men (8). It is important to highlight that it has been previously reported in a study from Latin American where girls had a lower frequency of hospitalization and disease severity than boys. Nevertheless, this report included not only COVID-19 patients but also COVID-19 related Multisystem Inflammatory Syndrome (MIS-C) patients, showing limitations in this report due to diverse clinical courses among both pathologies (4–14).

Several studies have shown that the median age of COVID-19 in pediatrics was between 7 and 11 years. In particular, a study in Argentina showed a median age of 7 years and infants under 12 months of age accounted for around 15% of the cases. Interestingly, our study shows a median age of 3 years and 62% of our cohort is under 5 years of age. It is according to data of a multinational experience from Latin America, where the median age of children with COVID-19 was 3 years (IQR 0.6–9 years) (2, 4–7, 11, 15, 16).

Common symptoms at onset of illness were fever and respiratory manifestations, associated with moderate and severe cases; followed by gastrointestinal manifestations (such as vomiting or diarrhea) present mainly in mild disease. It is worth noting that several reports show 45–65% of pediatric cases present fever (14–17). On the other hand, in a study from Latin America, 42/1,010 children had a diagnosis of acute abdomen based on clinical manifestations and 34/38 (89.7%) had a definitive diagnosis of acute apendicitis (18).

Different studies show that comorbidities may be risk factors for poor outcomes with severe infection and hospitalization. Our results align with these observations, showing that 21% of cases had underlying comorbidities and these cases were associated with moderate and severe cases. These comorbidities mainly included chronic pulmonary diseases such as asthma, bronchopulmonary dysplasia and recurrent wheezing. Interestingly, obesity and diabetes mellitus explain the predominant comorbidities in a multicentric study in the United States and onco-hematological diseases were predominant in another study from Argentina (2, 7, 12, 13).

Our results show that 20% of patients required hospital admission and patients with moderate or severe disease had a more prolonged length of stay (LOS). No association between age and hospitalization requirement was found in our study. Similar results were found in previous studies, where hospitalization frequency varied between 6 and 25% reaching 75% depending on the facility setting (14–19).

It is interesting to note that 6% of patients received antimicrobial treatment, mainly in moderate to severe cases due to possible bacterial co-infection. Another study in Latin America showed that 25% of patients received antimicrobial treatment and this was associated with severe cases of COVID-19 and MIS-C (20).

Regarding SARS-CoV-2 viral load, our study showed that higher amounts of viral RNA were found in samples of moderate to severe patients and in patients under 12 months. Several studies carried out in pediatric patients show a significant association between viral load and age, sex, comorbidities, severity of disease and hospitalization requirement. Differences were found in different studies addressing viral load and age. In particular, one study in Chile showed higher viral load in children under 10 years, in another study in New York this was associated with patients under 1 year, and finally in Chicago it was reported for children under 5 years (21–31).

Importantly, in our study no patient received a specific treatment, required admission to PICU nor required mechanical

TABLE 2 | Clinical and laboratory characteristics distributed by age group of patients ($n = 485$).

Variable	<1 months ($n = 106$)	13–60 months ($n = 191$)	61–120 months ($n = 108$)	>120 months ($n = 80$)	*P*
Males (*n*, %)	60 (57)	104 (54.4)	59 (54.6)	36 (45)	NS
Comorbidities (*n*, %)	13 (12)	46 (24)	27 (25)	16 (20)	NS
Evolution days (median, IQR)	1 (1–2)	1 (1–3)	1 (1–2.25)	2 (1–3)	NS
Hospitalization (*n*, %)	22 (20.8)	38 (20)	27 (25)	10 (12.5)	NS
Days of hospitalization (median, IQR)	4 (3-5)	4 (2.5–4)	3 (2.5–5)	5 (3.25–5.75)	NS
Supplementary oxygen (*n*, %)	8 (7.5)	22 (11.6)	14 (13)	5 (6.25)	NS
Antibiotic therapy (*n*, %)	9 (8.5)	7 (3.7)	7 (6.5)	5 (6.25)	NS
Days of antibiotic therapy (median, IQR)	4 (4–7)	7 (6–7)	5 (5–7)	7 (5–7)	NS
CT (*n*, %)					
<25 (high viral load)	26 (25%)	47 (25%)	23 (21%)	7 (9%)	<0.05
25–35 (intermediate viral load)	68 (64%)	125 (65%)	70 (65%)	56 (70%)	
>35 (low viral load)	12 (11%)	19 (10%)	15 (14%)	17 (21%)	
Days of oxygen supplementation (no mechanical ventilation)	2 (2–2.2)	2 (2–2.2)	2 (1–2)	2 (2–2)	NS

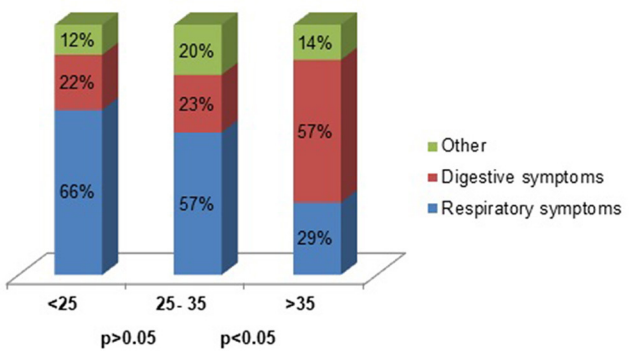

FIGURE 2 | CT PCR-SARS-CoV-2 and predominant symptoms ($n = 485$).

ventilation. No deaths were reported. These results are in line with reports from other healthcare settings, where critical cases and mortality are rarely observed. In Argentina, according to published data from the National Ministry of Health, two age groups of pediatric patients required PICU admission more frequently and showed higher mortality rates, children under 12 months and children between 10 and 14 years. It is important to note, that mortality in Argentina represents 0.5% of pediatric cases and was strongly associated with comorbidities (2). Despite the fact that our hospital covers a large part of Quilmes city population, it has no PICU facilities. Nevertheless, there were no COVID-19 pediatric cases involved in this study that required to be transferred to a PICU of another hospital. This shows that our analyzed population presented mainly mild to moderate disease with low frequency of severe cases and no critical disease.

Our study has several limitations. Firstly, due to the retrospective design, the loss of information in the clinical records is possible. The data collection has been carried out rigorously by the researchers, with a detailed record trying to reduce the risk of bias that this design represents. Secondly, due to the characteristics of the hospital in which it was carried out, it is possible that the population included is not completely representative of the pediatric cases in our country. However, since transfer to other more complex hospitals was not necessary, we can affirm that the patients included represent the best possible sample of the population served. It is important to note that one of the critical limitations of this study lies in considering the cycle threshold (CT) value as an indicator of viral load. In this regard, several variables might affect CT values such as collection technique, specimen type, sampling time, transport and storage conditions as well as nucleic acid extraction, viral RNA load, real time PCR efficiency and CT value determination method. Some of these limitations are thoroughly discussed by several authors and some modifications are proposed to reduce evaluation errors, such as normalization of CT values (32, 33). In spite of its limitations, correlations between SARS-CoV-2 Ct values and patient-related outcomes still have a clinical utility in the context the pandemic in a General Hospital in Latin America. Lastly, other important limitation is that we have no critical cases.

CONCLUSION

Our study shows that the viral load of SARS-CoV-2 in respiratory samples, determined indirectly and inversely by the cycle threshold, was significantly correlated with moderate to severe cases and with age, since infants younger than 12 months showed

higher amounts of RNA. Disease severity was associated with more frequent and longer hospitalization.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital Zonal General de Agudos Dr. Isidoro G. Iriarte. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MB designed the study. MB, SG, GC, MZ, LS, AC, and HF carried out and analyzed the data statistically and revised the manuscript. MB, SG, AC, and GC collected the information. All authors read and approved the final manuscript.

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Exhaled Aerosols in SARS-CoV-2 Polymerase Chain Reaction-Positive Children and Age-Matched-Negative Controls

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Background: Children and adolescents seem to be less affected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease in terms of severity, especially until the increasing spread of the omicron variant in December 2021. Anatomical structures and lower number of exhaled aerosols may in part explain this phenomenon. In a cohort of healthy and SARS-CoV-2 infected children, we compared exhaled particle counts to gain further insights about the spreading of SARS-CoV-2.

Materials and Methods: In this single-center prospective observational trial, a total of 162 children and adolescents (age 6–17 years), of whom 39 were polymerase chain reaction (PCR)-positive for SARS-CoV-2 and 123 PCR-negative, were included. The 39 PCR-positive children were compared to 39 PCR-negative age-matched controls. The data of all PCR-negative children were analyzed to determine baseline exhaled particle counts in children. In addition, medical and clinical history was obtained and spirometry was measured.

Results: Baseline exhaled particle counts were low in healthy children. Exhaled particle counts were significantly increased in SARS-CoV-2 PCR-positive children (median 355.0/L; range 81–6955/L), compared to age-matched -negative children (median 157.0/L; range 1–533/L; $p < 0.001$).

Conclusion: SARS-CoV-2 PCR-positive children exhaled significantly higher levels of aerosols than healthy children. Overall children had low levels of exhaled particle counts, possibly indicating that children are not the major driver of the SARS-CoV-2 pandemic.

Trial Registration: [ClinicalTrials.gov], Identifier [NCT04739020].

Keywords: exhaled aerosol, COVID-19 in children, COVID-19, aerosols, acute respiratory tract infection

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated pulmonary disease [coronavirus disease–2019 (COVID-19)] have caused millions of deaths worldwide (1). Since the virus' first description in December 2019, the pandemic has driven countries all around the globe to install various non-pharmaceutical interventions (NPIs) to mitigate the transmission of the virus. Prior to the global spread of the omicron variant, children and adolescents seemed to be less affected by the disease in terms of both, case numbers and severity (2, 3). However, the NPIs often included closing of schools, kindergartens and other educational and recreational groups for children and adolescents, which had a great impact on children's mental health and development (4).

In line with other viral illnesses, the symptomatology of a SARS-CoV-2 infection differs between children and adults. The most common presentation of COVID-19 in adults include early anosmia, followed by fever, dry cough and dyspnea (5–8). However, especially in older patients, it can cause severe pneumonia, which can lead to acute respiratory failure and fulminant sepsis with thromboembolic complications (5, 6). Children were more often found to be asymptomatic or mildly affected (9, 10). If symptoms were present, usually, headache, fatigue, fever, cough and sore throat were predominant, while hypoxemia and dyspnea remained rare (9–11). In general, illness duration was reported as shorter in children than in adults (10).

SARS-CoV-2 is spread through three main routes: (1) droplets, (2) aerosols, and (3) fomites (12–14). However, as per current research, the latter plays a lesser role in the spread of the virus (15–17). In contrast, aerosols seem to be a primary transmission route, as so called "super spreader" events describe clusters of cases especially in close proximity, but not necessarily with direct contact to an infected individual (16, 18–21). In the past, aerosols have been found to be important transmission routes for other bacteria and viruses; e.g., mycobacterium tuberculosis, influenza viruses and respiratory syncytial viruses (RSV) (22–25). In general, the term "aerosol" describes a suspension of particles with a gas (12, 26). According to the world health organization (WHO) definition, aerosols are suspensions containing particles <5 μm, while droplets contain particles >5 μm (27). However, bigger "droplet nuclei" can also evaporate into multiple smaller particles (27, 28).

In the mucosa of the upper respiratory tract, shear stress and airflow lead to surface instability, which breaks the fluid lining into small droplets and generates aerosols (29). In the lower respiratory tract, the surface instability stems from closing and reopening of collapsed terminal airways during tidal breathing, which also leads to the formation of small particles, exhaled as aerosols (30). Every human exhales a baseline amount of aerosols during tidal breathing, which is increased during speech, laughter or singing (31, 32). Certain infections might also increase the exhaled particle count, as some studies could show an increase in exhaled particles with SARS-CoV-2 infection in primates and individual humans (33, 34). When comparing exhaled particles between adults and adolescents, similar ranges for speaking were observed, but adolescents showed lower values when singing (35). Generally, it appears that exhaled particle counts increase with age (34), which could indicate that children pose a lower risk in viral transmission *via* aerosols (17). This might be due to the immature airway structure of children, which is composed of fewer alveoli and terminal bronchioles, as well as smaller breathing volumes and lower exhaled air speed (36, 37). Additionally, the cough push is less intensive in children compared to adults. However, the baseline exhaled particle count and changes secondary to respiratory tract infections are not well studied in children and adolescents.

As mentioned above, children and adolescents seem to be less affected by COVID-19, which appears to be due to (1) lower susceptibility to the virus and (2) a less severe disease course. The former might be explained with lower Angiotensin-Converting Enzyme 2 (ACE 2) expression in the mucosa, which leads to less entry-points for the virus (3, 38, 39). The latter is apparent through the high number of mild and asymptomatic cases, which often are only detected as incidental findings from screening tests (3, 40). This prospective study investigated the baseline level of exhaled particle count in healthy and SARS-CoV-2 infected children.

MATERIALS AND METHODS

Study Design

In a prospective observational cohort study, exhaled aerosol concentration and particle size were measured in SARS-CoV-2 polymerase chain reaction (PCR)-positive children and age matched healthy controls in the rhine main area and the city of Frankfurt, from January 18th to December 31st, 2021. Eligible participants were children and adolescents (6–17 years) with a SARS-CoV-2 PCR test prior to aerosol measurement.

Before recruitment into the study, detailed verbal and written information was provided for all participants and their legal guardians. With all patients and caregivers, the aims and risks of the study were discussed in detail. Prior to the start of the measurements, written consent, and, if appropriate, assent was obtained from all participants and their legal guardians. The investigation was approved by the Ethics Committee of the Goethe University Frankfurt (number 20–1001) and was registered under the ClinicalTrials.gov (Identifier: NCT04739020). The study was supported by a grant of Palas (Karlsruhe, Germany).

Participants

The participants were recruited in two ways: Firstly, children admitted to the Children's Hospital of the Goethe-University Hospital, Frankfurt, Germany, with SARS-CoV-2 PCR test, as part of the routine admission screening. Secondly, in cooperation with local physicians, SARS-CoV-2 PCR-positive children in the rhine main and Frankfurt city area were contacted, and, if the caregivers agreed, aerosol measurements were conducted in their homes. All participants were tested for SARS-CoV-2 *via* PCR prior to aerosol measurement. To account for differences between pre- and post-pubertal participants, it is a common practice to analyze pediatric data before and after the age of

12 years. Therefore, a sub-analysis in the age groups 6–11 and 12–17 years was conducted. Subjects were excluded from the study, if unable to participate in aerosol measurement, or if they and/or their legal guardian could not understand the extend and consequences of the study.

Study Procedures

Medical and Clinical History

A standardized questionnaire was used to record medical history, including preexisting medical conditions and medications. In addition, to obtain a clinical history, all participants together with their guardians were asked about acute symptoms of a viral illness in the context of SARS-CoV-2 infections. Those symptoms included fever, cough, shortness of breath, loss of taste or smell, sore throat, muscle pain, diarrhea and vomiting.

For every participant, height and weight were recorded and the body mass index (BMI), as well as the weight-for-height z-score were calculated. The z-score represents the number of standard deviations above or below a certain reference mean or median value. It is recommended by WHO to use z-scores to compare weight and height in smaller children (41).

Aerosol Measurement

In order to measure the exhaled aerosols, an aerosol spectrometer was used to count and size the exhaled particles (Resp-Aer-Meter, Palas GmbH, Karlsruhe, Germany). Particles in the size range of 0.15–5.0 μm with sizing resolution of 16 channels/decade were detected. To count the particles, an optical sensor creates a defined optical measurement volume using a polychromatic light source. The exhaled particles travel through this volume, generating a scattered light pulse. From the quantity and intensity of this light pulse, the particles' size and quantity can be determined.

For each measurement, a sterile sampling kit was used. The participants wore a nose clip, while breathing into a mouth piece with normal tidal breathing. The mouthpiece was connected with a T-piece, to a HEPA filter, which prevented particles from the surrounding air to mix with the exhaled particles, and to a heated hose, which was connected to the measurement device (**Supplementary Figure 1A** and **1B**). After about 1 min of tidal breathing, the remaining ambient aerosols were washed out from the hose and measuring device and the participants exhaled particle measurement was started. Each measurement lasted for about 1–1.5 min. Afterward, the results were displayed as a graphical curve and in numerical form (**Supplementary Figure 2**).

Spirometry

Using a hand-held device (Asthma Monitor® AM; VIASYS Healthcare GmbH, Höchberg, Germany), spirometry measurements were conducted with every participant according to the American Thoracic Society (ATS) and the European Respiratory Society (ERS) recommendations (42). The following parameters were obtained: Peak Expiratory Flow (PEF) and Forced Expiratory Volume in the first second (FEV1). To account for the influence of age, weight, and sex on FEV1, the FEV1% pred (FEV1 percentage of the predicted value) was calculated.

Outcomes

Primary aims of this investigation were to compare the exhaled particle counts in SARS-CoV-2 PCR-positive and age-matched-negative children and adolescents and to measure the baseline exhaled particle count in healthy children and adolescents. As secondary outcomes, change in exhaled particle concentration with age, sex, lung function, height, weight, z-score and BMI were assessed and the possible correlation between symptoms of viral illness and exhaled particle concentration measurements were examined.

Statistical Analysis

For statistical analysis, GraphPad Prism 5.01 (GraphPad Software, Inc.) and R 4.0.4 were used. All values were presented as median and range for numeric data and as percentage for count data.

The Wilcoxon-Mann-Whitney *U*-Test was used to test for group differences in numeric data and chi-squared test was used for count data. A p-value of < 0.05 was considered statistically significant.

The sensitivity and specificity of exhaled particle count measurements was assessed using Receiver Operating Characteristic (ROC) analysis. Correlations between cycle threshold (Ct) values and exhaled particle count measurements were calculated *via* Spearman correlation.

RESULTS

Patient Characteristics

In total, 162 children and adolescents were analyzed. Of those, 39 participants were tested positive for SARS-CoV-2 *via* PCR and 123 participants were tested negative. The 39 PCR SARS-CoV-2 positive children and adolescents were compared to age-matched SARS-CoV-2 PCR-negative controls. Patient characteristics are shown in **Table 1**. Overall, the characteristics of the SARS-CoV-2 PCR-positive and age-matched -negative children were not statistically different. Both groups had the same median age of 12 years ($p = 0.412$) and had the same slight male predomination (59.0 vs. 41.0%). The median BMI and z-score in the SARS-CoV-2 PCR-positive group were lower than in the SARS-CoV-2 negative group, with 17.2 vs. 19.8 kg/m^2 ($p = 0.051$) and −0.2 vs. 1.0 ($p = 0.035$), respectively.

Medical and Clinical History

Pre-existing medical conditions were present in 15.4% (6/39) of participants in the SARS-CoV-2 PCR-positive group and 30.8% (12/39) in the SARS-CoV-2 PCR-negative group (**Table 1**). The most common pre-existing medical conditions were asthma (2.6 vs. 15.4%), diabetes (0 vs. 7.7%) and allergies (7.7 vs. 2.6%).

Overall, in the SARS-CoV-2 PCR-positive group, 48.7% (19/39) had typical symptoms of a viral illness. The following symptoms were reported: cough in 35.9% (14/39), loss of taste/smell and sore throat both in 15.4% (6/39), shortness of breath in 7.7% (3/39), fever in 5.1% (2/39), and muscle pain in 2.6% (1/39). No participants in this group reported diarrhea

TABLE 1 | Characteristics of polymerase chain reaction (PCR) severe acute respiratory syndrome coronavirus 2 (SARS)-CoV-2 PCR-positive children and -negative age-matched controls.

	SARS-CoV-2 PCR-positive (*n* = 39)	SARS-CoV-2 PCR-negative (*n* = 39)	Total (*n* = 78)	*p*-value
Sex				
Female	16 (41.0%)	16 (41.0%)	32 (41.0%)	1.000
Male	23 (59.0%)	23 (59.0%)	46 (59.0%)	
BMI (kg/m^2)				
Median	17.2	19.8	18.6	0.051
Range	12.1–29.7	13.5–32.4	12.1–32.4	
Z-Score				
Median	−0.2	1.0	0.1	0.035
Range	−4.2–1.9	−3.0–2.4	−4.2–2.4	
Age (years)				
Median	12.0	12.0	12.0	1.000
Range	6.0–17.0	6.0–17.0	6.0–17.0	
FEV1 (%pred.)				
Median	80.5	85.0	83.2	0.066
Range	31.1–169.8	42.0–156.0	31.1–169.8	
Ct value				
Median	22	>40.0		
Range	17.7–31.5			
Comorbidities				
None	33 (84.6%)	27 (69.2%)	60 (76.9%)	0.178
Allergy	3 (7.7%)	1 (2.6%)	4 (5.1%)	0.615
Diabetes	0 (0%)	3 (7.7%)	3 (3.8%)	0.240
Respiratory disease	2 (5.1%)	7 (17.9%)	9 (11.5%)	0.154
Neurological disease	1 (2.6%)	1 (2.6%)	2 (2.6%)	1.000

p-Values for differences in SARS-CoV-2 PCR-positive and -negative participants are derived from Wilcoxon-Mann-Whitney U-Test for numeric data and from chi-square test for count data.

or vomiting as acute symptoms. In the SARS-CoV-2 PCR-negative group, 10.3% (4/39) reported acute symptoms: 5.1% (2/39) reported each diarrhea, vomiting and sore throat, 2.6% (1/39) reported cough. No participants in this group reported fever, shortness of breath, muscle pain or loss of taste/smell as acute symptoms.

Aerosol Measurements

Exhaled Particle Count

When looking at the exhaled particle counts of all children and adolescents (SARS-CoV-2 PCR-positive and -negative), the median was 211/L (0–6955/L).

When comparing the exhaled particle count in the SARS-CoV-2 PCR-positive and age-matched -negative groups, the median was significantly higher in SARS-CoV-2 PCR-positive children and adolescents (355/L [81–6955/L]) than in SARS-CoV-2 PCR-negative participants (151/L [1–533/L]; $p < 0.001$, **Figure 1**). When looking at the different age groups, in the group of children 6–11 years old, there was a significant difference between SARS-CoV-2 PCR-positive and -negative children, with a median exhaled particle count of 287/L (86–6955/L) and 145/L (0–533/L; $p = 0.023$), respectively. Furthermore, in the group of adolescents, 12–17 years old, a significant difference in exhaled particle counts could be found between the two groups (396.0/L [81–3982/L] vs. 171/L [0–502/L]; $p = 0.004$).

When separating the SARS-CoV-2 PCR-positive and -negative groups by age at a cut-off of age 12, within the two groups, there was no significant difference in exhaled particle counts. Within the PCR-positive groups, the median particle counts in children 6–11 years old and 12–17 years old were 287/L (86–6955/L) and 396/L (81–3982/L; $p = 0.777$), respectively. In the SARS-CoV-2 PCR-negative groups, the median particle counts in children 6–11 years old and 12–17 years old were 145/L (0–533/L) and 171/L (0–502/L; $p = 0.955$), respectively.

No significant difference of exhaled particle counts was found due to sex within the SARS-CoV-2 PCR-positive ($p = 0.461$) and -negative ($p = 0.710$) group. In addition, there was no correlation between exhaled particle counts and BMI ($p = 0.370$), z-score ($p = 0.572$), height ($p = 0.189$) or weight ($p = 0.185$).

When correlating the Ct value of each PCR test to the exhaled particle count measurements, there was a significant, negative correlation (Spearman correlation, r: −0.249; $p < 0.001$; **Figure 2**).

Receiver Operating Characteristic Analysis of Aerosol Concentration

A ROC analysis was conducted to determine the accuracy of the exhaled particle count measurements in detecting SARS-CoV-2 PCR -positive individuals (**Figure 3**). The analysis of all age-matched children showed an area under the curve (AUC) of 0.75. When separating the participants by age, the AUC was higher in adolescents, aged 12–17 years (AUC 0.76), compared to younger children, aged 6–11 years (AUC 0.73).

Spirometry

In the spirometry measurements, there was no difference between the two groups. In the SARS-CoV-2 PCR-positive and -negative group, the median PEFs were 271 L/Min (106–505 L/Min) and 286 L/Min (123–505 L/Min; $p = 0.961$), respectively. The median FEV1s were 2.3 L/s (0.9–3.8 L/s) and 2.4 L/s (0.7–4.1 L/s; $p = 0.270$), respectively. In addition, the median FEV1% pred were 80.5% (31.1–169.8%) and 85.0% (42.0–156.0%; $p = 0.066$), respectively.

DISCUSSION

In general, transmission of bacteria and viruses *via* aerosols has been apparent for several years (24, 25). As demonstrated in various studies, aerosols are the primary transmission route of SARS-CoV-2 (16, 18–21). The virus was found to retain structural integrity for more than 12 h in aerosol suspensions (43). While children and adolescents have been reported to transmit the virus (2, 44, 45), they do not seem to be the main driver of viral spread, especially prior to the omicron wave of infections that started in December 2021; e.g., a transmission from adult to child has found to be more common than vice versa, in multiple settings (39, 46). Viral respiratory illnesses can spread rapidly from one child

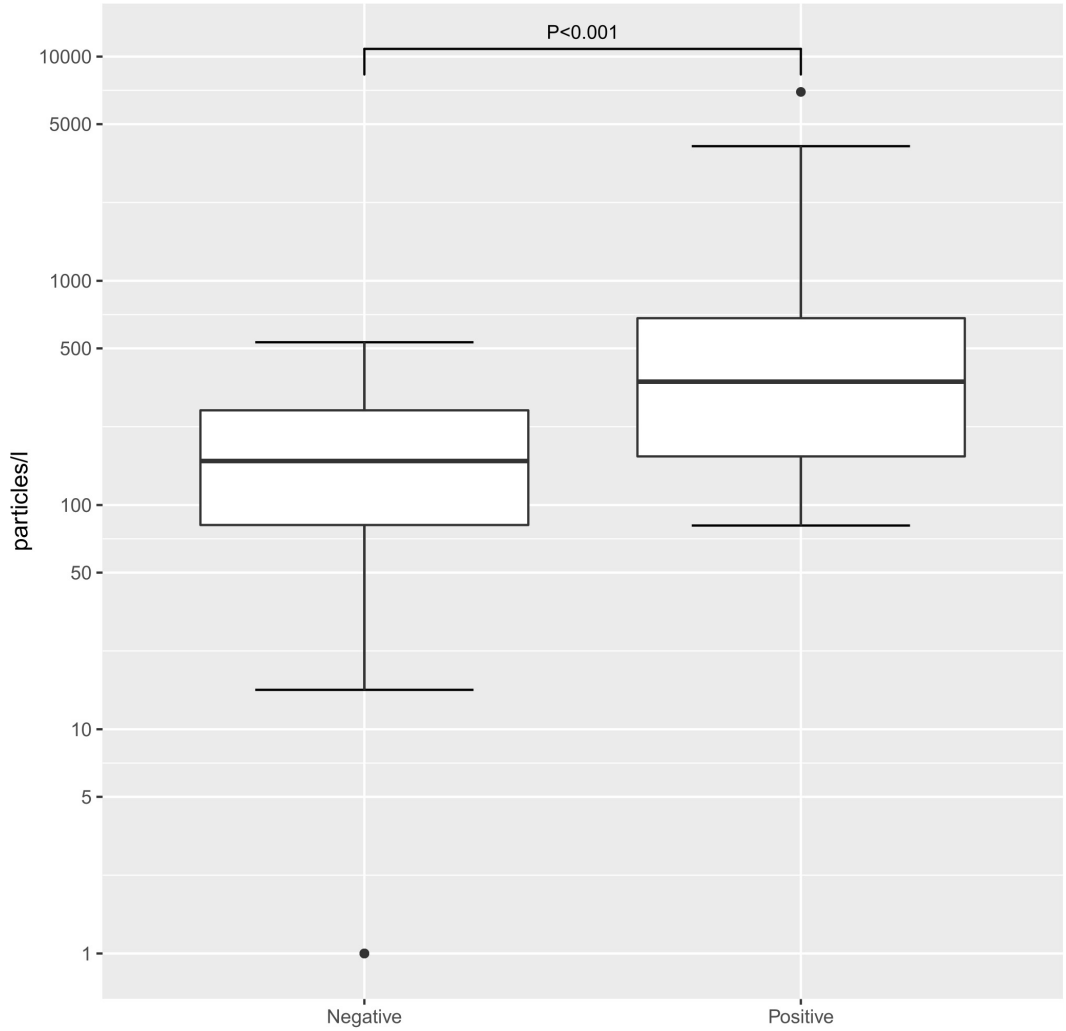

FIGURE 1 | Exhaled particle counts in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR)-positive and -negative children. Exhaled particle counts in particles/L Exhaled particle counts in particles/L, displayed on a logarithmic scale.

to another, for example, in a study from Chu et al. during RSV season, after the first case of RSV was found in a childcare group, within 1 week, the virus was transmitted to 50% of children who were in the same room (47). However, in the case of SARS-CoV-2, children seemed to be less contagious than adults (48).

One reason for decreased contagiousness is probably that children emit a lower number of exhaled particles. This might be due to anatomical differences in the immature airway structure of children, which consists of fewer alveoli and terminal bronchioles, which are thought to be the origin of aerosol production (36, 37). In line, the current study demonstrated that children and adolescents overall emitted only a small amount of exhaled particles when compared to adults (49). In addition, in all healthy controls (SARS-CoV-2 PCR-negative) a median exhaled particle count of 170.0/L (68.0–298.5/L) was measured. Edwards et al. found an increase in exhaled particles with age (34). In the present study, when the healthy controls were divided into age groups (cut-off 12 years), there was no difference between the two groups. The overall lower baseline exhaled particles might be contributing to the finding that children, sometimes even with higher viral loads than adults, seem to be less symptomatic and less contagious (3, 17, 40).

Another reason for possibly decreased contagiousness of children and adolescents might be decreased susceptibility to the virus and less severe symptoms (3, 48). As SARS-CoV-2 enters the human body through ACE 2 receptors, the lower expression of those receptors in children likely plays a role in the decreased susceptibility (3, 38, 39). In addition, an immunity against other seasonal coronavirus contributes to the multitude of asymptomatic cases (3, 40). Nevertheless, in coherence with our previous findings and the findings of Edwards et al., SARS-CoV-2 PCR-positive children and adolescents produced significantly more exhaled particles than SARS-CoV-2 PCR-negative controls (34, 49). In addition, we found a significant negative correlation

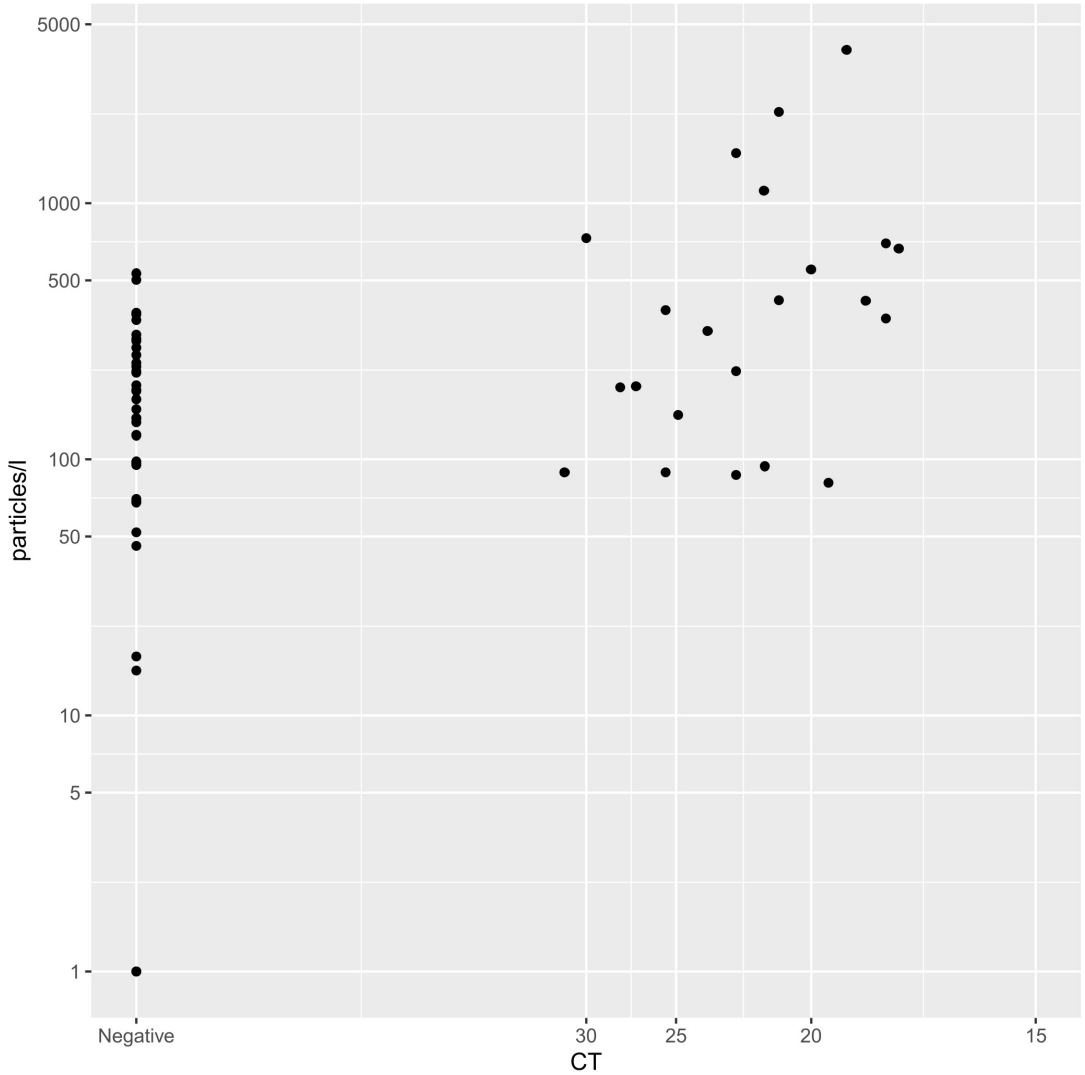

FIGURE 2 | Spearman correlation of CT-value and exhaled particle count. Displaying the correlation between Ct-value (x-axis) and exhaled particles (y-axis).

of Ct value and exhaled particle count, showing higher exhaled particle counts in lower Ct values. While statistically significant, this increase of exhaled particle counts in SARS-CoV-2 PCR-positive children and adolescents was not as substantial, as seen in our previous work with adults (49). A possible explanation for this observation being lower rates of symptomatic participants, as only 48% of all SARS-CoV-2 positive children were symptomatic and those had only mild to moderate symptoms. However, the lower overall aerosol production in children and adolescents most likely contributes to this difference aswell.

Apart from the aforementioned correlation between exhaled particle counts and age, Edwards et al. also found the BMI to be a confounding factor (34). This correlation was not found in the present cohort, however, as BMI is not a very reliable measurement in children, height, weight and z-score were analyzed, but did also not show any relationship. Furthermore, there was no significant association with lung function.

The present findings suggest that measurements of exhaled particle counts do not have sufficient validity to be used as a testing tool in children (AUC 0.75). However, previous studies (35, 46, 48) and this investigation, show that they are an important research tool and might help to determine which NPIs are most appropriate for mitigation of viral transmission in different age groups. From the present results, children might be less likely to spread SARS-CoV-2 *via* aerosol transmission and, in addition, seem to be less symptomatic. However, this has to be confirmed in further studies.

This study has several limitations to be considered. Although the PCR testing and exhaled particle count measurements were conducted as close to each other, as possible, they could not be performed simultaneously. Considering the changes in viral load throughout the course of the infection (50), it is possible that the results were affected by this time window. Especially,

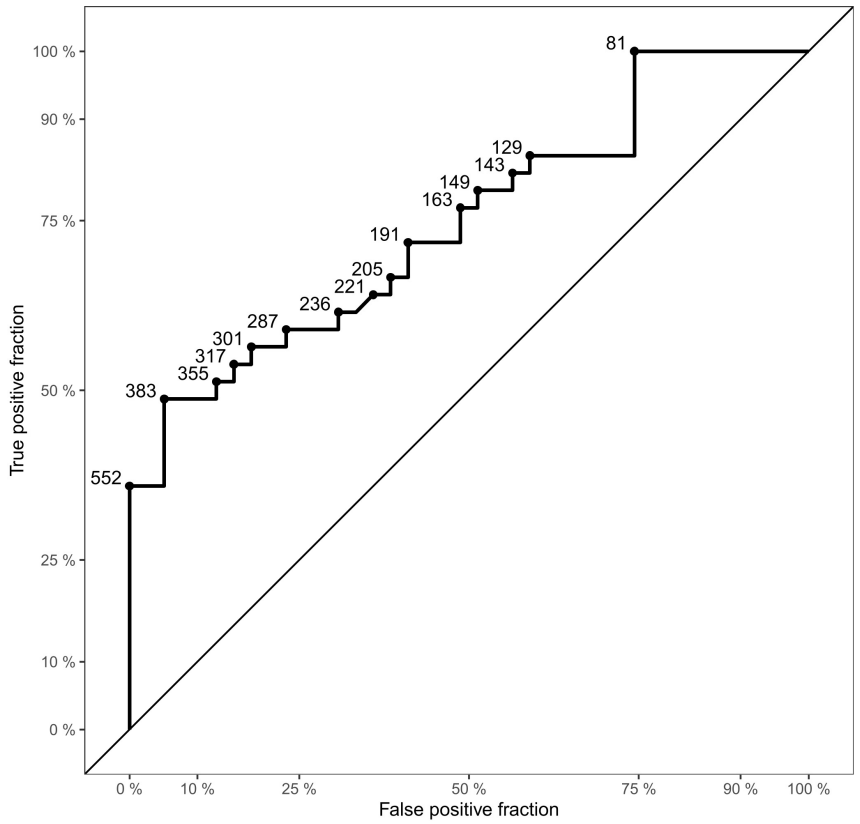

FIGURE 3 | Receiver Operating Characteristic (ROC) curve of the dataset. Displaying sensitivity (true positive fraction) in the *y*-axis and 1-specificity (false positive fraction) on the *x*-axis. Points on the curve show examples of cut-off values (aerosol particles per liter).

because only a one-time measurement in each participant was conducted, the dynamics of aerosol emission during an infection is unclear. Edwards et al. (34) found an exponential rise of particle counts in primates up to day 7 of infection, with a steep decline thereafter. Longitudinal measurements are necessary to assess, if this is also true for humans. Furthermore, only asymptomatic and mildly to moderately symptomatic children and adolescents were included in our study. Further studies should assess whether similar quantities of aerosol particles are produced by children and adolescents with more severe infection.

In conclusion, significantly higher counts of exhaled particles could be found in SARS-CoV-2 PCR-positive children and adolescents, when compared to -negative controls. In addition, the median exhaled particle count of all children and adolescents was lower than previous measurements in adults. An improved understanding of exhaled particle counts in various age groups might help to determine feasible and reasonable mitigation strategies for viral transmission.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Goethe University Frankfurt. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DG, HD, MH, MW, and SZ conceived and designed the trial and SZ was the principal investigator. DG, HD, LH, TL, AL, and EU collected trial data and involved with data curation. DG and MH, were responsible for the formal data analysis. DG, MH, TL, LH, AL, EU, HR, and SZ verified the underlying data supporting the findings of this manuscript. All authors had full access to the full data set in this study and critically reviewed and approved the final version.

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Goethe University. Palas GmbH reviewed the study data and final manuscript before submission, but the authors retained editorial control.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.941785/full#supplementary-material

Supplementary Figure 1 | Aerosol Resp-Aer-Meter **(A)** structure and **(B)** function.

Supplementary Figure 2 | Sample measurement of Resp-Aer-Meter.

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Traditional Chinese Medicine in Treating Children With Coronavirus Disease 2019: A Scoping Review

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Coronavirus disease 2019 (COVID-19) is currently widely spread across the world. Traditional Chinese Medicine (TCM) plays an important role in the overall treatment process. As a special group of population, the treatment outcome of children with COVID-19 has attracted much attention. Our study summarizes the current situation of TCM treatment of children with COVID-19. The results showed that TCM displayed a positive role in the treatment process, and that no significant adverse reactions were found. Our findings provide analytical evidence for the efficacy and safety of TCM participation in the treatment of COVID-19 in children.

Keywords: COVID-19, children, traditional Chinese medicine, syndrome differentiation, efficacy and safety

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INTRODUCTION

In the past 2 years, coronavirus disease 2019 (COVID-19) has repeatedly emerged as an acute respiratory infectious disease. Children have their own unique physiological and pathological characteristics, and they respond differently to the virus compared with adults. Studies have shown that patients with mild COVID-19 may be less likely to be seroconverted in children than in adults at the same viral load (1). It has also been shown that long-term humoral immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children last longer even after asymptomatic infection than in adults (2). At present, the emergence of SARS-CoV-2 variants has caused the increasing prevalence in children around the world (3–6). More research is urgently needed on the long-term relationship between COVID-19 and children (7). Clinicians have tried a variety of therapeutic regimens and constantly summarized the experience during the process. Corresponding treatment guidelines have been formulated around the world. Among the regimens, Traditional Chinese Medicine (TCM) is mentioned in diagnosis and treatment plans in China (8–11), and it is widely used and shows unique advantages. In March 2022, the World Health Organization (WHO) Expert Meeting on Evaluation of TCM in the Treatment of COVID-19 was held, and it was noted that participation of TCM could reduce the aggravation rate of mild and moderate patients and shorten the duration of viral shedding and hospital stay. The safety of TCM treatment is similar to that of conventional treatment (12). Currently, most studies have focused on describing the TCM efficacy evidence of COVID-19 in adults, and there is less evidence about children.

Studies have shown that children with COVID-19 have a variety of initial symptoms, some have fever and respiratory symptoms (13, 14), some have digestive tract symptoms (15, 16), and

Abbreviations: COVID-19, Coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCM, Traditional Chinese Medicine; WHO, World Health Organization; RCT, randomized controlled trial.

some patients have no obvious clinical symptoms but only have fatigue (17). In the course of disease development, there are more changes in symptoms. TCM can flexibly respond to changes in symptoms according to syndrome differentiation. Children act as a special group of people, and a comprehensive and systematic evaluation of the efficacy of TCM is an urgent problem to be solved at present. The results can provide a reference for doctors to guide drug use and for the formulation of a TCM prevention and treatment of COVID-19 policy for children. Our study summarizes the current situation of TCM treatment of COVID-19 in children.

METHODS

Search Strategy

The following databases were searched from establishment to 2 December 2022: CNKI, Wanfang, SinoMed, PubMed, Cochrane Library, and Embase. The MeSH terms include COVID-19, children, and TCM. We also manually searched for studies that met our inclusion criteria from other sources that were not included in the aforementioned databases. Two researchers (Duan N. F. and Liu B.) independently selected eligible studies. Studies in any language were retrieved.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (1) meeting the diagnostic criteria of COVID-19 diagnosis and treatment protocol: version 4–8 (18–22), (2) randomized controlled trials (RCTs), retrospective cohort studies, and retrospective clinical observational studies, (3) patient age < 18 years old, (4) the intervention measures are integrated Traditional Chinese and Western medicine or TCM alone, and (5) the primary outcome is clinical outcome, and the secondary outcomes can include with or without hospitalization, time to viral shedding, adverse reactions, and time to symptom resolution. The exclusion criteria were as follows: (1) guidelines, reviews, network pharmacology, and basic experimental research, (2) suspected case research, (3) no treatment regimens were described, and (4) missing primary outcome data.

Data Extraction and Risk of Bias Assessment

According to standard information extraction tables, two researchers (Duan N. F. and Liu B.) independently extracted the data. Throughout the process, disagreements were resolved by discussion or by involving another researcher (Lu C.). The basic information extracted from the articles included authors' names, publication year, published region, type of study design, date of illness onset, virus detection results, number of cases, sex, age, medical history, epidemiological history, type of clinical classification, syndrome differentiation, symptoms, tongue image, treatment regimens, course of treatment, outcome indicators, and adverse reactions.

We conducted a risk of bias assessment of the included RCT studies. Two reviewers (Duan N. F. and Li X. N.) independently assessed the risk of bias in each study using the criteria outlined in the Cochrane Handbook (2019). Any disagreements were resolved by discussion or by involving another author (Lu C.). The risk of bias was assessed according to the following domains: (1) random sequence generation, (2) attrition bias, (3) allocation concealments, (4) blinding of participants and personnel, (5) blinding of outcome assessment, (6) incomplete outcome data, (7) selective outcome reporting, and (8) other biases. Each potential source of bias was graded as high, low, or unclear, providing a quote from the study report and a justification of our judgment in the "risk of bias" table. In the table, red represents high risk, yellow represents unclear risk, and green represents low risk. We also added notes in the table when information on the risk of bias was related to unpublished data or correspondence with a trial author. When evaluating treatment effects, we considered the risk of bias in studies that contributed to the outcome.

Data Synthesis and Analysis

The Review Manager 5.2 software was used to produce the risk of bias summary figure. Categorical variables were expressed as counts and percentages. Continuous variables were described using median with interquartile range. All the statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.).

RESULTS

Search Results and Study Characteristics

A total of 558 studies were identified using the search strategy and included 72 from CNKI, 121 from Wanfang, 91 from SinoMed, 134 from PubMed, 0 from the Cochrane Library, and 140 from Embase. Of these, 218 duplicate studies were excluded, and 319 studies were excluded after abstract review. Ultimately, 21 studies involving 406 cases were included. Among these, there were 19 retrospective studies (23–41) and 2 RCTs (42, 43). The flowchart of the screening process is presented in **Figure 1**.

Retrospective Clinical Study

Twelve studies were published in 2020 and 7 in 2021. The distribution area covers 13 provinces in China. The age range is mainly 7–18 years old. There were 147 men and 99 women. Clinical classification was 13 asymptomatic, 133 mild, and 81 moderate types. The main symptoms were fever and cough, and some cases were accompanied by nausea and vomiting, diarrhea, constipation, and other gastrointestinal symptoms. Another part of the cases did not show clinical symptoms during the medical process. A very small number of children had a previous medical history. Detailed characteristics of the studies are presented in **Table 1**.

Treatment Regimens Analysis

Fifteen studies used integrated TCM and Western medicine treatment regimens, and 4 studies used TCM treatment regimens alone. TCM treatment is based on syndrome differentiation according to different case characteristics. The cases of 6

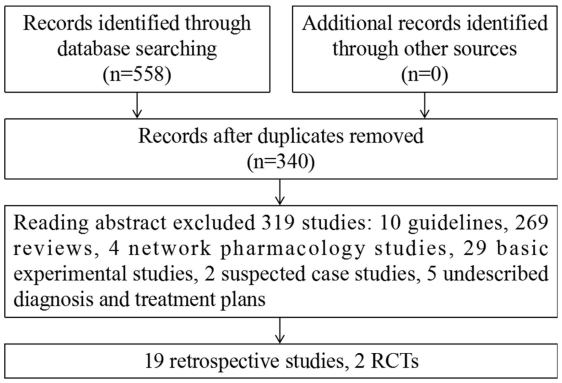

FIGURE 1 | Flowchart of including and excluding studies.

studies had damp-heat syndrome, 3 showed cold-damp syndrome, and 3 had lung/spleen Qi-Yin deficiency syndrome in the later stage of the disease. The remaining studies did not clearly specify the type of syndrome differentiation. The main symptoms of damp-heat syndrome include fever, cough, headache, chest tightness, heavy body, poor appetite, loose stool, reddened tongue, and yellow greasy tongue coating. The main symptoms of cold-damp syndrome include fever, cough, nasal congestion, runny nose, pale tongue, and white greasy tongue coating. The main symptoms of lung/spleen Qi-Yin deficiency syndrome include fever, cough, fatigue, pharyngoxerosis, reddened tongue, and thin tongue coating.

The most commonly used type of drug is TCM decoction. Cold-damp syndrome uses *Qingfei Paidu Decoction* more often. If the damp-heat syndrome is more damp than heat, *Huoxiang Zhengqi Powder*, *Sanren Decoction*, *Shenling Baishu Powder* are used, if the heat is more than damp, *Maxing Shigan Decoction* will be added. For the Lung-spleen Qi-Yin deficiency syndrome, the most commonly used medicine is *Yupingfeng Powder*, *Shashen Maidong Decoction,* and *Shengmai Powder*. The commonly used Chinese patent medicines include *Lianhua Qingwen capsule*, *Xiaoer Chaigui Tuire granules*, *Pudilan Xiaoyan oral liquid*, etc. A part of the cases used TCM injection for adjuvant treatment.

Western medicine treatment regimens are mainly antiviral drugs combined with antibiotics and symptomatic supportive treatment according to disease situation. Among the 19 studies included, the number of antiviral drug use times is: 13 studies using interferon, 6 studies using Ribavirin, 4 studies using Arbidol, 6 studies using Lopinavir/Ritonavir, and 4 studies using Oseltamivir. The number of antibiotic use times is: 5 studies using azithromycin, 3 studies using cephalosporin antibiotics, 2 studies using penicillinase antibiotics, and 1 study using vancomycin. The number of immunotherapy use times is: 3 studies using gamma globulin and 1 study using human immune globulin. The symptomatic support treatment programs include hormonotherapy, regulating intestinal flora, defervescence, anticoagulation, oxygen inhalation, etc. The details are presented in **Table 1**.

Treatment Outcomes

The clinical outcomes of all the cases included in the 19 studies were cured and discharged. Ten studies counted the length of hospital stay, which ranged from 9.5 to 32 days. Among them, there were 6 studies using integrated traditional Chinese and Western medicine regimens, and the length of hospital stay was 15, 9.5, 21, 15, 10.8, and 19.2, respectively. There were 4 studies using TCM regimens alone, and the length of hospital stay was 15.9, 13, 15-32, and 21 days, respectively. Nine studies counted the time of viral shedding, which ranged from 1 to 32.6 days. Among them, there were 7 studies using integrated traditional Chinese and Western medicine regimens, and the time of viral shedding was 16, 1–6, 12.5, 23, 14.7, 4–21, and 7–23 days, respectively. There were 2 studies using TCM regimens alone, and the time of viral shedding was 15.2–32.6 and 19 days, respectively. One study counted the time to fever resolution, and the time was 1.76 days, and the treatment regimen was TCM treatment alone. One study counted the time of stool viral shedding, and the time was 15.5 days, and the treatment regimen was traditional Chinese and western medicine.

In the above studies, some of the time outcome indicators were calculated in the mean value, some in the median value, and the data cannot be combined and analyzed, so the results are presented in the form of tables. The details are presented in **Table 1**.

Prospective Clinical Study

The 2 studies were published in 2021 and 2022, respectively. The detailed characteristics of the studies are presented in **Table 2**.

One study included 100 children with damp-heat syndrome of COVID-19. The treatment group used Yishen Jianpi massage combined with Western medicine, and the control group used Western medicine alone. The observation period was from admission to discharge. After treatment, symptom remission rate was higher in the treatment group than in the control group, and the mean disease duration of the two groups was 3.76 ± 2.21 and 4.66 ± 2.18 days, respectively. No adverse reactions occurred in either group. The study has shown that adding pediatric massage can shorten the course of the disease and relieve the symptoms (42).

One study included 60 children with cold-damp syndrome of COVID-19. The treatment group used thunder-fire moxibustion plus *Qingfei Paidu Decoction*, and the control group used *Qingfei Paidu Decoction* alone. After 5 days of treatment, cough disappearance rate and immunoglobulin levels were higher in the treatment group than in the control group, and there was no significant difference in fever disappearance rate. The study has shown that adding thunder-fire moxibustion can accelerate the improvement of part symptoms and improve body immunity (43).

The assessment of risk of bias (**Figure 2**) showed that 2 studies implemented randomized grouping. There was no mention of allocation concealment and blinding. With regard to incomplete outcome data and selective reporting, the 2 studies reported the results according to preset outcome indicators and were rated as

TABLE 1 | Characteristics of 19 retrospective studies on children with COVID-19.

Study	Sex		Age (Y)	Medical history	Type			TCM syndrome	Symptoms	TCM			Western medicine			Outcomes			Dis charged
	M	F			Asymptomatic	Mild	Moderate			Decoctions	CPMs	Others	Antiviral drugs	Antibiotics	Others	Period (D)	Fever resolution (D)	Viral shedding (D)	
Ge et al. (23); Hubei	0	1	12.5	–	0	0	1	D-H syndrome, L-S Qi-Yin deficiency syndrome	Fever, cough, headache, chest tightness, heavy body, poor appetite, loose stool	Sanren Decoction, Shenling Baizhu Powder, Maxing Shigan Decoction, Yupingfeng Powder	–	–	IFN, Ribavirin	Ceftezole	Defervescence	15	–	–	Yes
Yuan et al. (24); Fujian	50	37	8.4 (3–13)	G6PD deficiency1, Surgery of ASD repair 1, Hyperthyroidism 1, Chronic tonsillitis 1,Allergic rhinitis 1	0	49	38	D-H syndrome	No symptoms 24, fever 59, chill 3, sweating 3, nasal congestion 14, runny nose 9, headache 2, cough 22, sore throat 2, throat itching 1, dry mouth 5, hyposphraesia 1, hypogeusia 2, poor sleep 3, constipation 12 and diarrhea 1	Shangjiao Xuanbi Decoction, Shengjiang Powder, Maxing Shigan Decoction, Shashen Maidong Decoction, Shengmai Powder	–	Herbal plaster andgargle	–	–	Symptomatic support treatment	15.9	1.76	–	Yes
Hu et al. (25); Jilin	11	10	10 (1.6–17)	–	2	13	6	D-H syndrome, C-D syndrome	No symptoms 11, cough 6, fever7, diarrhea 2, bitter mouth 1, pharyngeal discomfort 1, and nasal congestion and runny nose 1	Hanshiyi Decoction, Hanshi Zufei Decoction, QFPD Decoction, Xuanfei Baidu Powder, Wenfei Huashi Decoction, Jieji Qingre Decoction, Pingwei Powder, Wenbu Pishen Decoction	–	Xi yanping, Xuebijing Injection	Arbidol, Ribavirin	Ceftriaxone sodium	Anticoagulation, Oxygen inhalation	–	–	16 ± 7.19	Yes
Liu et al. (26); Shanxi	0	2	8 (7–9)	–	1	1	0	C-D syndrome	Cough phlegm	QFPD Decoction	–	–	IFN, LPV/r	–	–	9.5	–	–	Yes

(Continued)

TABLE 1 | (Continued)

Study	Sex		Age (Y)	Medical history	Type			TCM syndrome	Symptoms	TCM			Western medicine			Outcomes			Dis charged
	M	F			Asymptomatic	Mild	Moderate			Decoctions	CPMs	Others	Antiviral drugs	Antibiotics	Others	Period (D)	Fever resolution (D)	Viral shedding (D)	
Luo et al. (27); Henan	0	3	6 (0.5–8)	–		3		–	–	TCM Decoction	–	–	IFN, LPV/r	–	–	–	–	–	Yes
Shang et al. (28); Shanghai	1	0	7	–	0	0	1	D-H syndrome, L-S Qi-Yin deficiency syndrome	Fever, chills, light cough, nausea	Huangqin Qingre Lishi Mixture, Jinbai Mixture	Xiaoer Chaigui Tuire Granule, Pudilan Oral liquid, Xingpi Yanger Granule, Huaiqihuang Granule, Huangqi Granule	–	IFN	–	Triviable bifidobacteria	21	–	–	Yes
Chen et al. (29); Hubei	3	4	8 (2.6–14)	2 cases	2	0	5	–	Fever 4, cough and sputum 5	TCM Decoction	–	–	Arbidol	–	–	–	–	–	Yes
Chen et al. (30); Hubei	25	7	6.9 (0.2–15.1)	Surgery of ASD repair 1, Ophthalmic strabismus surgery 1, ALL 1	0	30	2	Yidu Yufei Syndrome	Fever, chill, cough, muscle soreness, headache, chest tightness, sore throat, vomiting, abdominal pain, poor appetite, diarrhea, convulsions	TCM Decoction	-	–	IFN, Oseltamivir	Azithromycin	Gamma globulin	–	–	–	Yes
Si et al. (31); Guizhou	1	0	13	–	0	1	0	C-D syndrome	–	Mahuang Jiazhu Decoction, Huoxiang Zhengqi Powder, Shengjiang Powder	–	–	–	–	Symptomatic support treatment	13	–	–	Yes
Yang et al. (32); Yunnan	4	1	7.5 ± 5.2	–	2	2	1	–	Cough 1, pharyngeal discomfort 1, myalgia 1	QFPD Granule, Qingyun Jiere Decoction, Xuanfei Baidu Decoction	–	–	–	–	Symptomatic support treatment	15	–	15.2 ± 4.4	Yes Yes

(Continued)

TABLE 1 | (Continued)

Study	Sex		Age (Y)	Medical history	Type			TCM syndrome	Symptoms	TCM			Western medicine			Outcomes			Dis charged
	M	F			Asymptomatic	Mild	Moderate			Decoctions	CPMs	Others	Antiviral drugs	Antibiotics	Others	Period (D)	Fever resolution (D)	Viral shedding (D)	
	7	4	9.8 ± 5.2	Hepatitis B 1	1	7	3	–	Fever 5, cough 3, fatigue 1, hyposphraesia or hypogeusia 1, nasal congestion 1, runny nose 2, pharyngeal discomfort 6		–	–	–	–		32	–	32.6 ± 7.0	
Ji et al. (33); Hubei	3	1	0.9 (0.76–10)	–	0	1	3	–	Fever 2, cough 1, nasal congestion 1, short of breath 1	Maxing Shigan Decoction, Shegan Mahuang Decoction, Xiaochaihu Decoction, Wuling Powder	–	–	IFN, Arbidol, Oseltamivir	Azithromycin	Human immune globulin, Splenamino peptide, Budesonide, Magnesium isogglycyrrhiza, Prototype glutathione, High-dose vitamin C	15	–	–	Yes
Zhan and Bai (34); Hubei	3	3	8.5 (0.5–11)	–	1	4	1	–	Fever 4, cough 3, sore throat 3, sneezing 1	TCM Decoction	BairuiGranule, LHQW Capsule, Fufang Yuxingcao syrup	Tanreqing Injection	IFN	Potassium amoxicillin-clavulanate, Piperacillin sodium tazobactam, Cephalosporins, Norvancomycin, Azithromycin	Sodium fructose diphosphate, Gammaglobulin, Ibuprofen, Oxygen inhalation, Atomization, Adjustment of intestinal flora	–	–	1–6	Yes
Tian et al. (35); Tianjin	2	0	9.5 (9–10)	–	0	0	2	Shiwen Syndrome 2, Shixie Kunbiao Syndrome 1, Fengwen Xibiao Syndrome 1	Fever 2, cough 2, fatigue 1, epistaxis 1, dry stool 1	Huoxiang Zhengqi Powder, Yinqiao Powder, Maxing Shigan Decoction, Sanren Decoction	–	–	IFN	–	–	–	–	12.5	Yes
Hu et al. (36); Hunan	7	2	3.9 ± 3.7	–	0	9		–	Fever, cough, headache, dizziness, poor tolerance	COVID-19 Prevention 2 Decoction	LHQW Capsule	–	IFN, Paramivir, Albidol, Oseltamivir, LPV/r	Ceftazidime	Methyl prednisolone, Human immunoglobulin	10.8 ± 6.5	–	–	Yes

(Continued)

TABLE 1 | (Continued)

Study	Sex		Age (Y)	Medical history	Type			TCM syndrome	Symptoms	TCM			Western medicine			Outcomes			Dis charged
	M	F			Asymptomatic	Mild	Moderate			Decoctions	CPMs	Others	Antiviral drugs	Antibiotics	Others	Period (D)	Fever resolution (D)	Viral shedding (D)	
Chen et al. (37); Guangzhou	1	0	7	–	1	0	0	–	Asymptomatic	Zhengyang Decoction, QFPD Decoction, Toujie Quwen Decoction	–	–	IFN, LPV/r, Ribavirin	Cefixime, Azithromycin	–	–	–	23	Yes
He et al. (38); Shanghai	6	5	5.5 (1.8–10)	Hepatic insufficiency after BA 1	0	6	5	Yidu Fanwei Syndrome, D-H syndrome, L-S Qi-Yin deficiency syndrome	Early stage: fever 7, cough 9, nasal congestion and runny nose 4; middle stage: cough 5, poor appetite 1, loose stool 1	Huangqin Qingre Lishi Mixture, Shegan Mixture, Jinbai Mixture	Pudilan oral liquid	–	IFN, Ribavirin	Azithromycin	Ibuprofen, Ambroterol, Bifidobacteria	10–29 (19.2 ± 7.2)	–	7–27(14.7 ± 7.2) (nasopharynx); 8–26(15.5 ± 7) (Feces)	Yes
Zhang et al. (39); Yunnan	2	2	6.42 (3–10)	–	1	2	1	Xiefan Shaoyang Syndrome	Fever 3, cough 3, sore throat 1, fatigue 2	Xiaochaihu Decoction	–	–	IFN, LPV/r, Ribavirin	Antibiotics	–	–	–	4–10 (case 1, 2, 3); 21 (case4)	Yes
Cao et al. (40); Shandong	20	17	0–18	–	2	8	11	–	Fever 13, cough 7, diarrhea 1, chills 1, headache 1, nasal congestion 2, runny nose 2, sore throat 1, vomiting 2, abdominal pain 1, constipation 1	TCM Decoction	LHQW Capsule	Tanreqing Injection	IFN, Oseltamivir, Ribavirin, LPV/r	–	Propoglobulin plus coenzyme Q10 was combined with vitamin C	–	–	7–23	Yes
Liu et al. (41); Hubei	1	0	1.92	–	0	0	1	–	Diarrhea	TCM Decoction	–	–	-	–	Symptomatic support treatment	21	–	19	Yes

"M," male; "F," female; "Y," year; "D,", day; "TCM," Traditional Chinese Medicine; "CPMs," Chinese patent medicines; "G6PD deficiency," glucose-6-phosphate dehydrogenase deficiency; "Surgery of ASD repair," surgery of atrial septal defect repair; "ALL," acute lymphoblastic leukemia; "Hepatic insufficiency after BA," hepatic insufficiency after biliary atresia; "D-H syndrome," damp-heat syndrome; "L-S Qi-Yin deficiency syndrome," Lung/spleen Qi-Yin deficiency syndrome; "C-D syndrome," cold-damp syndrome; "IFN," interferon; "LPV/r," Lopinavir/Ritonavir; "LHQW Capsule," Lianhua Qingwen capsule; "QFPD Decoction," Qingfei Paidu decoction; "–," not mentioned in the original study.

TABLE 2 | Characteristics of 2 randomized control trials (RCTs) on children with COVID-19.

Study	Syndrome	Sex (M/F)	Age (Y)	Cases	Type (Mild/Moderate)	Treatment	Period (D)	Outcomes							
		T/C	T/C	T/C	T/C	T/C		Total effective rate (T/C)	Syndrome points	Fever disappearance rate	Cough disappearance rate	Immunoglobulin levels	Changes in the blood routine	Course (D)	ADRs
Li (43)	Cold-damp syndrome	17/13; 15/15	8.69 ± 2.37/ 9.23 ± 2.25	30/30	6/24; 9/21	Thunder-fire moxibustion plus Qingfei Paidu Decoction/Qingfei Paidu Decoction	5	83%/60%	The T was lower than C (P < 0.01)	No significant difference (P > 0.05)	The T was higher than C (P < 0.01)	The T was higher than C (P < 0.05 or P < 0.01)	No significant difference (P > 0.05)	–	No
Zhan et al. (42)	Damp-heat syndrome	31/19; 29/21	4.23 ± 3.15/ 5.33 ± 3.31	50/50	–	Yishen Jianpi massage combined with western medicine/western medicine	From admission to discharge	-	The T was lower than C (P < 0.05)	–	–	–	–	(3.76 ± 2.21)/(4.66 ± 2.18)	No

"M," male; "F," female; "Y," year; "D," day; "T," treatment group; "C," control group; "ADRs," adverse reactions; "–," not mentioned in the original study.

FIGURE 2 | Assessment of risk of bias in 2 trials.

low risk. The 2 studies showed no other obvious biases and were rated as low risk.

The above results reflect the positive role of TCM external methods in the treatment of children with COVID-19. However, the above two studies only described the random number table method. No blinding methods and allocation concealment methods were mentioned. We contacted the corresponding author by telephone and email to seek a detailed study process without a response. Therefore, the results of the two randomized controlled studies have a lower evidence level.

DISCUSSION

According to the analysis of the above studies, TCM has a high participation rate in the treatment of COVID-19 in children. The external treatment methods also displayed their unique advantages. No obvious adverse effects appeared during the treatment process.

COVID-19 is a viral infectious disease. The survival of SARS-CoV-2 is related to environmental temperature and humidity (44–46). Different climates have different temperatures and humidity. Take the climate of southern and northern China as an example; the Qinling Mountains-Huaihe River is the dividing line between the north and the south of China, and between the warm temperate climate and the subtropical climate. Due to climatic differences, temperature and humidity are higher in the south than in the north. Therefore, it can lead to different

disease characteristics. The most important feature of TCM is syndrome differentiation (47, 48). In the included studies above, damp-heat syndrome mostly appear in the south area (24, 38) and cold-damp syndrome mostly appear in the north area (25, 26). The main difference lies in the treatment of cold syndrome and heat syndrome. TCM theory has a treatment principle called "*Rezhe Hanzhi*, *Hanzhe Rezhi*" (cold prescriptions are used to treat the heat syndrome, and heat prescriptions are used to treat the cold syndrome). *Scutellaria baicalensis Georgi*, *Bupleurum Chinense (DC)*, and *gypsum* are the main representatives of heat-clearing medicine. Studies have shown that the antipyretic effect of *Scutellaria baicalensis Georgi* and *Bupleurum Chinense (DC)* is accomplished by regulating PGE2 and cAMP and inhibiting the synthesis or release of endogenous pyrogens TNF-α and β-EP (49, 50). The antipyretic effect of *gypsum* is mainly attributed to its trace elements, in which calcium, zinc, cadmium, cobalt, and lead directly participate in the antipyretic effect, and iron, copper and selenium play an indirect antipyretic role by regulating the immune system (51). *Ephedra sinica Stapf* and *Cinnamomi Ramulus Herb* are the main representatives of cold-dispelling medicine (52). *Luteolin*, the main active ingredient of *Ephedra sinica Stapf*, has strong anti-inflammatory, antiviral, and antibacterial effects, and it shows significant efficacy in treating severe acute respiratory syndrome (53). *Ephedra sinica Stapf* inhibited inflammatory response by reducing the amount of the airway inflammatory factors IL-3 and IL-4 in asthmatic rats (54). The antiviral effects of *Cinnamomi Ramulus volatile oil* and *Cinnamaldehyde* are reflected in reduction of proinflammatory cytokine release by regulating the proportion of T cell subsets, thus enhancing the immune defense system and alleviating lung tissue pathological damage (55). Cold and heat theory can be used as a bridge to understand the TCM theory.

Children of different age groups have different physiological and pathological characteristics. Children between 0 and 18 years old can be divided into baby period (birth to 1 year old), toddler period (1–3 years old), preschool period (3–7 years old), grade-schooler period (7–14 years old), and adolescent period (14–18 years old) (56–58). The studies above have shown that TCM can be used in the treatment of COVID-19 in children of all ages (23, 28, 33, 41), and that all children are cured and discharged from hospital, more or less, TCM treatment regimens have been added. The curative effect of TCM is certain, and no obvious adverse reactions occurred. The treatment of children with COVID-19 is still inexperienced. In the future, child cases should be collected in time, and treatment regimens should be continuously summarized and improved (59–61). TCM participates in the treatment of COVID-19 in children of all ages, which is a positive option in clinical practice.

Moreover, external treatment methods provide more options for the treatment of COVID-19 in children, and the advantages of external treatment methods have been gradually shown (62). External methods for children mainly include massage (63), acupuncture (64), herbal plaster (65), and so on. External treatment is mostly operated in the body surface, which can observe the patient's tolerance situation at any time, thus deciding whether to continue the treatment. Although the two studies included had lower levels of evidence, they provide a reference for external treatment of children with COVID-19. More high-quality clinical and experimental research should be carried out in the future.

At present, China is actively introducing to the world a continuous improvement practice experience in COVID-19 TCM diagnosis and treatment. The main measures include remote video exchange of treatment experience, donation of TCM materials, and support of TCM doctors to foreign countries. However, because of differences in national policies, systems, and cultures, the spread of TCM is limited to some extent (65). The number of English media reports on TCM has increased, and the global English audience has become more familiar with TCM, which has built a positive image of TCM (66). At present, the evidence of efficacy and safety of TCM treatment of COVID-19 is increasing. Therefore, the key point for TCM to be recognized is efficacy. Our study further adds evidence to the treatment of COVID-19 in children using TCM.

There are also some limitations to our study. Most of the included literature is retrospective studies, most of the studies have fewer cases, and the outcome indicators are calculated with different methods, which cannot objectively compare the advantages of TCM in treating COVID-19 in children. The included RCT studies were partially methodologically lacking, and there are no RCT studies treatment with oral TCM drugs. Therefore, more high-quality, well-designed, multicenter RCT studies are still needed to provide evidence for the efficacy and safety of TCM in treating children with COVID-19 in the future.

CONCLUSION

At present, TCM is widely used in treatment of children with COVID-19, and no obvious adverse reactions have occurred. Application of TCM is a positive option in clinical practice. However, most of the current studies are retrospective clinical observational studies, and the randomized controlled studies have lower levels of evidence. More high-level evidence is still needed to verify the effectiveness and safety of TCM treatment for COVID-19 in children.

AUTHOR CONTRIBUTIONS

CL, ND, and BL conceived and designed the review. ND, XL, and BL wrote the initial draft. YX, YM, and LL were responsible for literature checking and result discussion. All authors contributed to the final version of the manuscript.

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Viral infections in hospitalized children in Germany during the COVID-19 pandemic: Association with non-pharmaceutical interventions

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Background: Non-pharmaceutical interventions (NPI) during the COVID-19 pandemic aimed at prevention of SARS-CoV-2 transmission also influenced transmission of viruses other than SARS-CoV-2. The aim of this study was to describe and compare the burden of common viral respiratory and gastrointestinal infections in children admitted to Berlin University Children's Hospital (BCH) before and during the COVID-19 pandemic at different levels of public NPI measures.

Methods: In this retrospective study, we analyzed the frequency of detection of common human respiratory and gastrointestinal viruses from January 2016 through January 2022 in all patients admitted to BCH. We compared virus detection before and during the COVID-19 pandemic at different levels of public NPI measures.

Results: The frequency of detection of seasonal enveloped and non-enveloped viruses [Boca-, Corona-, Influenza-, Metapneumo-, Parainfluenza-, Rota-, and Respiratory Syncytial Viruses (RSV)] was diminished during the COVID-19 pandemic, whereas detection rates of non-seasonal viruses (Rhino-/Entero-, and Adenoviruses) were stable during the pandemic. After withdrawal of major NPI measures, we observed an out of season surge of the detection rates of Boca-, Corona-, Parainfluenzaviruses, and RSV. In contrast, no increased detection frequency was observed for Influenza-, Metapneumo-, and Rotaviruses as of January 2022.

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Conclusion: Corona-, Boca-, Parainfluenzaviruses, and RSV returned as frequently detected pathogens after withdrawal of major NPI measures. The out of season rise might be attributed to an "immune-debt" due to missing contact to viral antigens resulting in waning of population immunity during the COVID-19 pandemic.

KEYWORDS

COVID-19, pandemic, child, Bocavirus, RSV, non-pharmaceutical interventions (NPI), respiratory virus infection, gastrointestinal infection

Introduction

Since 2020 countries around the world focused on the mitigation of effects of the COVID-19 pandemic to their population (1). The paramount goal was to reduce morbidity and mortality by infection with SARS-CoV-2. During 2020, when there were no vaccines against SARS-CoV-2 available, countries imposed non-pharmaceutical interventions (NPI) that aimed at the prevention of virus transmission. In Germany, mass gatherings were prohibited, working remotely became mandatory where feasible, schools and kindergartens were closed as well as retail shops, restaurants, bars, and cultural institutions (Figure 1A). Restrictions on border crossing and associated quarantine measures led to a decrease in traveling activity (Figure 1B). Use of face masks became mandatory in public areas, buildings and public transport. Quarantine became obligatory in cases of infection or an increased risk of infection with SARS-CoV-2. Parts of these measures were temporary and corresponded to dynamics of the local and countrywide incidence of SARS-CoV-2 infection (Figure 1C). Other measures were still in place in January 2022, such as the use of face masks in public buildings and public transport.

On December 21, 2020, the European Medicines Agency (EMA) recommended the first COVID-19 vaccine for authorization in Europe and within 1 week vaccination was commenced in Germany (2). From then on until mid-2021 NPI measures were gradually lifted, corresponding to vaccination rates and to dynamics of incidence of infection with SARS-CoV-2 and hospital capacities. COVID-19 immunization was initially not approved for children. Only a small part of the pediatric population, adolescents from an age of 12 years, was immunized against SARS-CoV-2 in Germany, starting on August 16, 2021. The fear of transmission of SARS-CoV-2 among children attending school or kindergarten led to governmental directives enforcing closures of schools and kindergartens, in contrast to workplaces (3). In Berlin, kindergartens were finally reopened in May 2021, in-classroom teaching for all students was commenced after the 2021 summer holidays, in August 2021.

While NPI measures during the COVID-19 pandemic focused on the prevention of the transmission of SARS-CoV-2, it was likely that NPI measures also affected the transmission of other, airborne/droplet or fecal-orally transmitted viral pathogens. As major NPI measures were lifted in Germany during spring and summer of 2021, their preventive effect on infections other than SARS-CoV-2 might have been suspended too. This may have led to a recurrence of common viral infections in children and adolescents. Yet it was unclear whether the long period of NPI measures affected the population immunity to common pathogens and the presence of these pathogens in Berlin and Germany.

The aim of this study was therefore to determine the frequency of common airborne/droplet or fecal-orally transmitted viral infections in hospitalized children and adolescents at BCH, the largest children's university hospital in Germany. We compared the rates of detection of viral pathogens in hospitalized children and adolescents before and during the COVID-19 pandemic at different levels of public NPI measures.

Materials and methods

Study design

We undertook a retrospective longitudinal study to compare monthly rates of detection of common viral respiratory and gastrointestinal tract infections in children admitted to BCH before the COVID-19 pandemic and during the pandemic at different levels of public NPI measures. During the observation period from January 2016 to January 2022 admitted patients with symptoms of a respiratory tract infection regularly underwent diagnostic testing for respiratory viruses [human Bocavirus (BoV), Coronaviruses 229E, NL63, HKU1, and OC43 (CoV), Influenzaviruses A, B (InV), human Metapneumovirus (MPV), Parainfluenzaviruses 1-4 (PIV), Respiratory Syncytial Viruses A, B (RSV), Rhino-/Enteroviruses (RV/EV)], as well as SARS-CoV-2 (since 2020) using a PCR-based Respiratory Pathogen Panel. Admitted patients with diarrhea regularly underwent testing for Rotavirus (RoV) by an enzyme immuno assay (EIA) or by Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and Adenoviruses (AdV) by PCR. We analyzed all results obtained by Respiratory Pathogen Panel testing, all Rotavirus

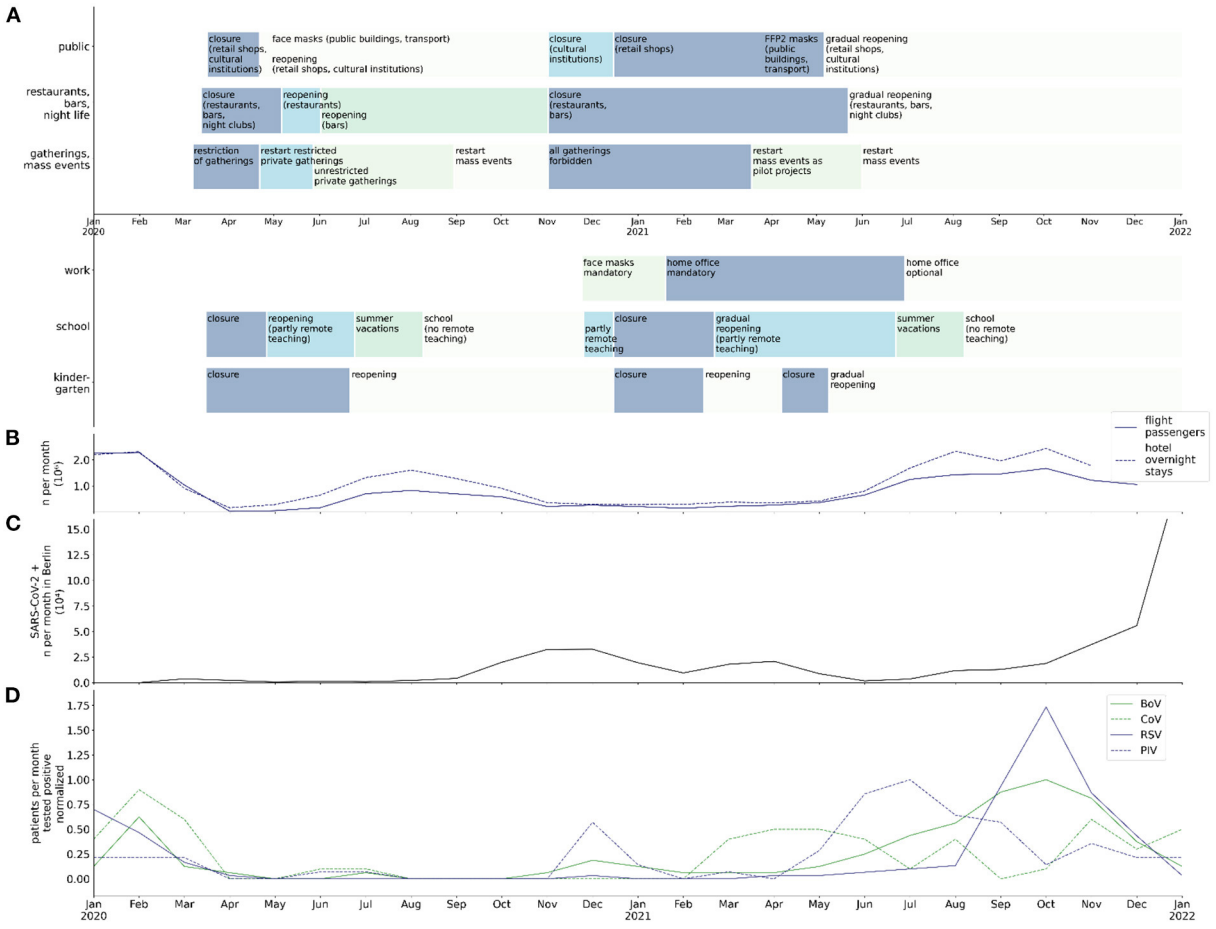

FIGURE 1
Chronology of NPI, traveling, SARS-CoV-2 detection rate in Berlin, and normalized virus detection rate at BCH between January 2020 and January 2021; **(A)** chronology of public regulations in public buildings/spaces, restaurants and night life, private and public gatherings, workplace, school, and kindergartens in Berlin; **(B)** numbers (10^6) of flight passengers and overnight stays in hotels per month in Berlin; **(C)** rate of SARS-CoV-2 infections (10^4 per month) in Berlin; **(D)** max-normalized rate of infections (per month) with BoV, CoV, RSV, and PIV in hospitalized patients at BCH.

EIA/RT-PCR and Adenovirus PCR results of all children (age < 18 years), regardless of gender and comorbidities, admitted to BCH during the observation period. As the first period of social and hygiene restrictions ("lockdown") in Berlin began on March 14, 2020, we defined the beginning of April 2020 as the beginning of the pandemic period in terms of our analyses concerning the frequency of common respiratory and gastrointestinal tract viruses.

Sampling and laboratory analyses

The decision for diagnostic testing of inpatients was made by the physician in charge based on the patient's respiratory or gastrointestinal symptoms. Outpatients were not tested. In spontaneously breathing patients, an oropharyngeal swab was performed and analyzed. In mechanically ventilated patients we analyzed tracheal secretion or an oropharyngeal swab. Nucleic acids were isolated from 200 µl respiratory sample material using the MagNA Pure 96 instrument (Roche Diagnostics, Germany) according to the manufacturer's instructions. Amplification and detection of nucleic acids from respiratory pathogens were performed using the qualitative test NxTAG® Respiratory Pathogen Panel as well as NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 (NxTAG® RPP + SARS-CoV-2; Luminex Corporation, USA). The NxTAG workflow included multiplex RT-PCR and bead hybridization in a thermocycler (GeneAmp 9700, Applied Biosystems, USA) followed by read on the MAGPIX System (Luminex Corporation, USA). The limit of detection (LoD) titer for each of the NxRPP targets was defined as the lowest concentration at which ≥95% of the samples tested generated positive calls and was reported of 10^2 to 10^4 copies/ml. Detection of AdV DNA was performed after manual isolation of nucleic acids

from 200 µl stool suspension (QIAamp DNA Mini Kit, Qiagen, Germany) followed by real-time PCR according to Heim et al. (4) on LightCycler® 2.0 (Roche Diagnostics, Germany). The LoD of the AdV target in the in-house assay is 2,000 copies/ml. All positive results, whether weakly or strongly positive, were classified as a virus detection.

Data collection and analysis

Data were generated and analyzed using the instrument specific software, obtained results were stored in the laboratory information system and analyzed using Python 3.7 and its libraries (open source). We excluded all tests with an equivocal result. To avoid a possible bias by multiple testing during an ongoing infection of the same patient with the same virus, we included only the first positive PCR or EIA result of each virus from every 8 weeks interval of each patient. We excluded all viruses detected <60 times during the observation period from our analyses. This excluded Adenovirus (throat swab PCR) and Norovirus (stool PCR). Flight passenger statistics in Berlin were derived from the official website of Berlin Brandenburg airport. Statistics on overnight hotel stays in Berlin were derived from destatis, the official website of the Federal Statistical Office (Statistisches Bundesamt) of the Federal Republic of Germany.

We used Shapiro-Wilk test to test for normal distribution of monthly virus detection rates. Only the monthly detection rate of RV/EV detection followed normal distribution. Consequently, we used a two-tailed student's *t*-test to test for statistical significance of the observed results concerning RV/EV. To test for statistical significance of the observed results concerning all other viruses, we used a two-tailed Mann-Whitney *U* test. Ethical review or approval were not required for this study.

Results

Detection of respiratory viral pathogens

The total number of analyzed respiratory samples was 5,237, total number of patients tested for respiratory viruses was 3,159. The mean total monthly number of molecular biology testing of throat swabs from hospitalized patients was 66.2 before the COVID-19 pandemic, 68.5 from the beginning of the pandemic through May 2021, and 109.3 from June 2021 to January 2022 (Figure 2A).

Viral positive respiratory specimens per month before and during COVID-19 pandemic

The pre-pandemic monthly number of throat swabs of hospitalized patients tested positive by RT-PCR showed a seasonal behavior of BoV, CoV, InV, MPV, and RSV detection (Figure 3). It showed a two peaked seasonality of PIV detection. In contrast, there was no seasonality of RV/EV. From the beginning of the COVID-19 pandemic in Germany through May 2021, several viruses were no longer detected by RT-PCR or the number of monthly positive throat swabs was substantially diminished (Figure 3). This applied to InV (whole year, monthly mean: 2.92 pre-pandemic, 0 during pandemic, $p = 0.001$), MPV (whole year, monthly mean: 2.88 pre-pandemic, 0.07 during pandemic, $p < 0.001$), and RSV (whole year, monthly mean: 6.22 pre-pandemic, 0.29 during pandemic, $p = 0.007$). The seasonality of detection of these viruses in throat swab samples by RT-PCR was lost. The seasonal peak of monthly BoV detection was decreased during the pandemic (season Nov-May, monthly mean: 4.48 pre-pandemic, 1.33 during pandemic, $p = 0.002$). The seasonal peak of CoV detection was delayed by 3 months. The number of RV/EV positive throat swabs did not significantly decrease during the pandemic (whole year, monthly mean: 16.69 pre-pandemic, 14.07 during pandemic, $p = 0.15$), although there were periods with a decreased detection rate of RV/EV in April/May 2020 and January/February 2021. The overall monthly number of PIV detection resulting from molecular diagnostics was reduced during the COVID-19 pandemic (whole year, monthly mean: 3.75 pre-pandemic, 1.21 during pandemic, $p < 0.001$) and it did not peak between April and June 2020. However, there was a peak of PIV detection frequency in December 2020, which corresponded to the seasonal peak before the COVID-19 pandemic.

Viral positive respiratory specimens per month after withdrawal of major NPI measures

While the monthly rates of BoV, PIV, and RSV detected in throat swabs by RT-PCR decreased during the COVID-19 pandemic, they increased after withdrawal of major NPI measures (Figure 3). From June through November 2021, the monthly rates of throat swabs tested positive for BoV, CoV, PIV, and RSV significantly exceeded corresponding pre-pandemic monthly detection rates (BoV: 1.3 vs. 10.5 mean monthly positives, $p < 0.001$; CoV: 0.5 vs. 2.7 mean monthly positives, $p = 0.008$; PIV: 3.1 vs. 8.3 mean monthly positives, $p = 0.007$; RSV: 0.4 vs. 19.2 mean monthly positives, $p < 0.001$). Moreover, the increase in the monthly rate of throat swab samples tested positive for BoV, PIV, and RSV between June and November 2021 was temporally unrelated to these viruses' pre-pandemic seasonality. In the case of PIV, the maximum detection rate per month after withdrawal of major NPI measures was comparable to the pre-pandemic maximum (pre-pandemic: 14; July 2021: 14). In the case of RSV, it even exceeded the pre-pandemic maximum (pre-pandemic: 30; October 2021: 51; Figure 1D). Interestingly, several months after withdrawal of major NPI measures, we observed an increase of MPV detection frequency by molecular testing that was in line with the pre-pandemic seasonality of MPV detection (June 2021 to January 2022: $p =$

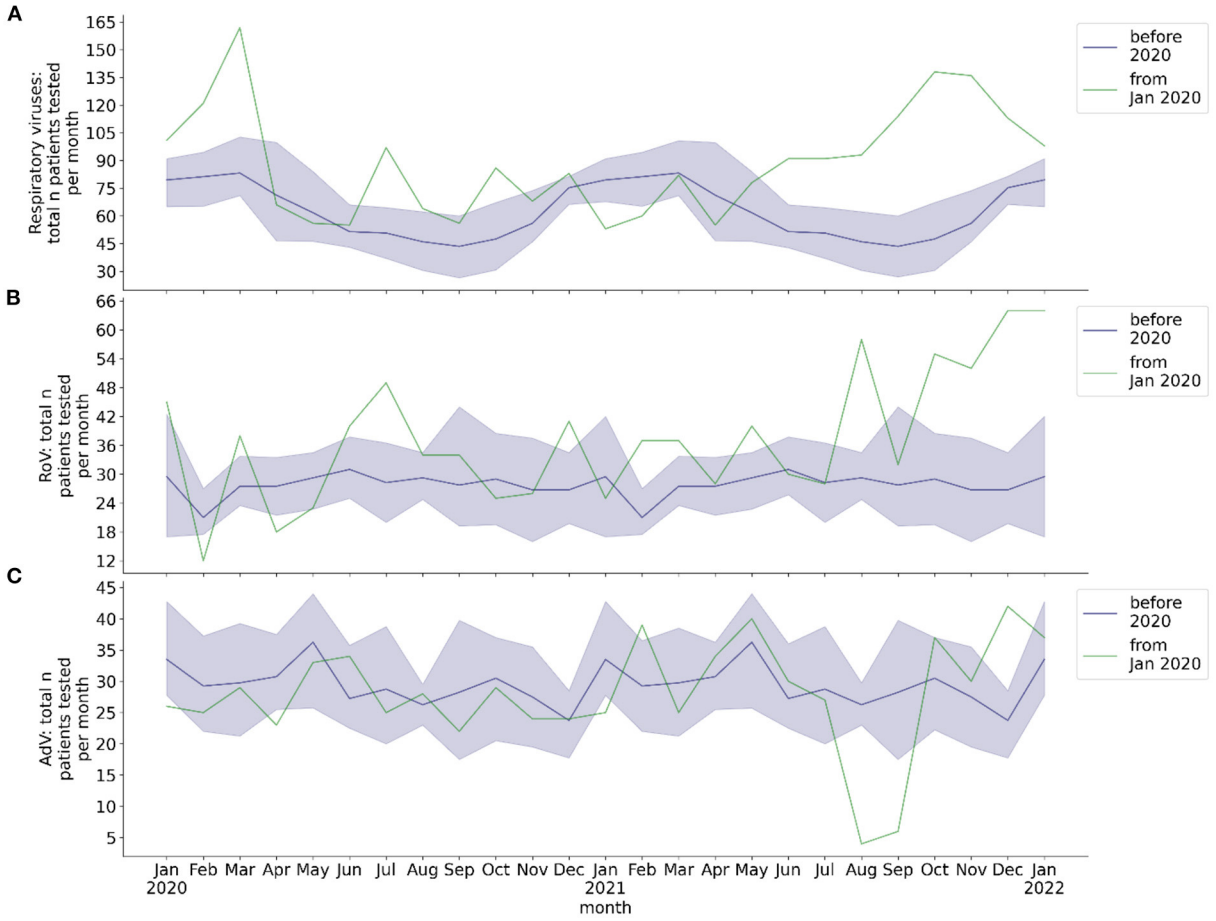

FIGURE 2
Total monthly number of hospitalized patients at BCH tested for respiratory viruses and for RoV and AdV, respectively. Blue line: mean 2016-2019, blue shade: 95% confidence interval 2016-2019, green line: absolute number per month from 2020, calendar months 2016-2019 are shown as a historical reference and correspond to calendar months, not to year on x-axis. **(A)** Total number of patients per month with molecular biology testing of respiratory samples for respiratory viruses; **(B)** total number of patients per month with EIA or molecular biology testing of stool sample for RoV; **(C)** total number of patients per month with molecular biology testing of stool sample for AdV.

0.90). In comparison to pre-pandemic detection rates, RV/EV detection rate was decreased between July and October 2021 (14.7 vs. 2.5 mean monthly positives; $p < 0.001$), during a period of high detection rates of other respiratory viruses, but showed a peak thereafter which exceeded the pre-pandemic maximum detection rate per month (pre-pandemic: 27; November 2021: 35). We observed no increase of monthly detection of InV in throat swab samples after withdrawal of NPI measures.

Detection of rotavirus/adenovirus in stool

The total number of analyzed stool samples was 4,450, total number of patients tested for gastrointestinal RoV/AdV infection was 1,540. The mean total monthly rate of stool EIA/PCR was 28.0 for RoV and 29.2 for AdV before the COVID-19 pandemic, 32.6 for RoV and 28.9 for AdV from the beginning of the pandemic through May 2021, and 47.9 for RoV and 26.6 for AdV from June 2021 to January 2022 (Figures 2B,C). The monthly rate of stool samples tested positive for RoV by EIA/PCR before the pandemic exhibited a seasonal behavior, whereas the rate of stool samples tested positive for AdV by PCR did not (Figure 4). No seasonality of the rate of RoV infection was observed during the COVID-19 pandemic. During the pandemic, the mean monthly rate of patients with stool EIA/PCR tested positive for RoV decreased (season January to May: 0.29, $p = 0.017$), compared to the pre-pandemic period (season January to May: 2.30). In contrast, the monthly number of patients with AdV detected in stool samples by PCR did not decrease during the COVID-19 pandemic (whole year: 1.78 pre-pandemic, 1.29 beginning of pandemic through May 2021, $p = 0.29$). We observed no increase in the monthly detection rate of RoV and AdV by EIA/PCR of stool samples from June 2021 (withdrawal of NPI measures) to December

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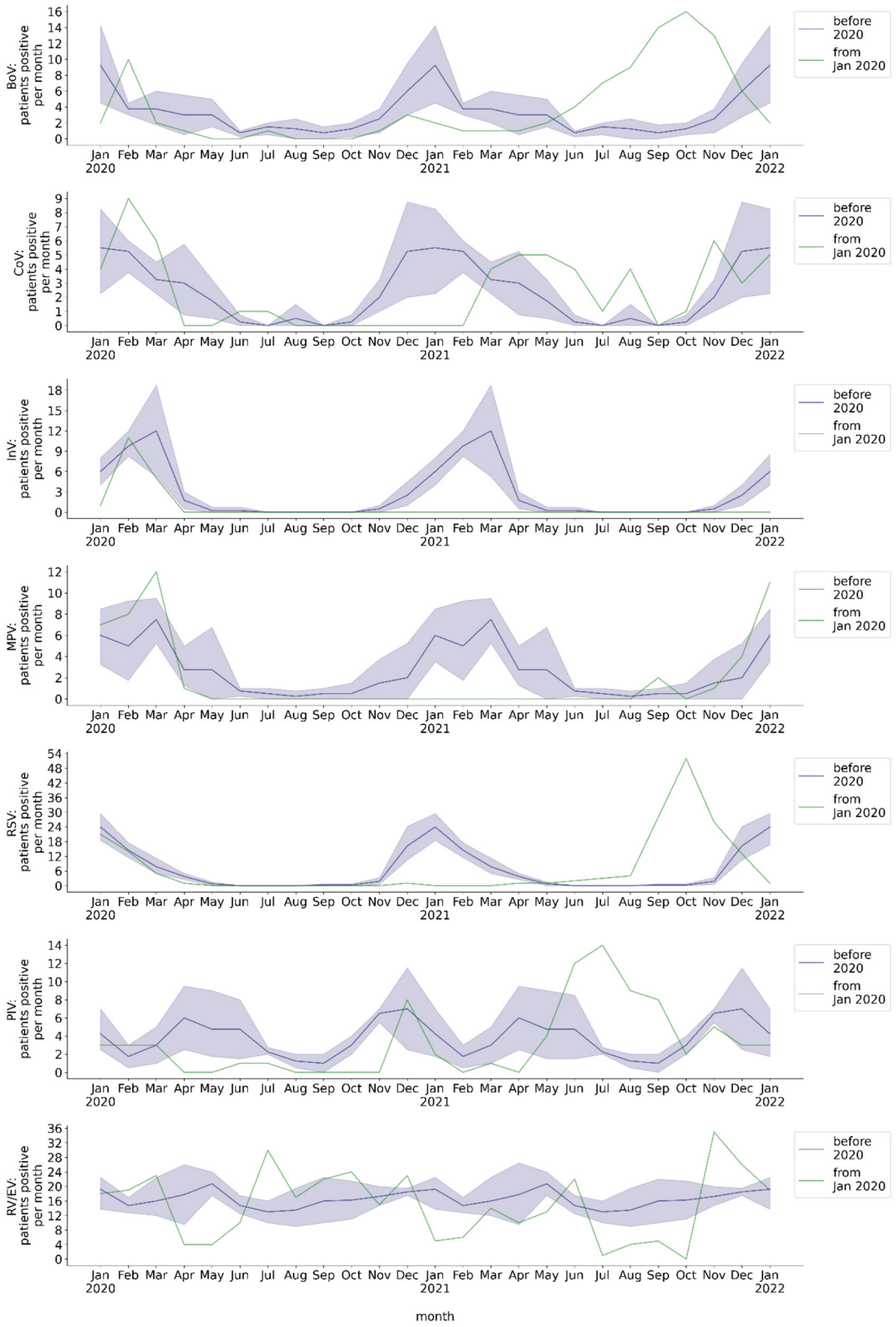

FIGURE 3
Monthly number of hospitalized patients at BCH positively tested for respiratory viruses. Blue line: mean 2016-2019, blue shade: 95% confidence interval 2016-2019, green line: absolute number per month from 2020, calendar months 2016-2019 are shown as a historical reference and correspond to calendar months, not to year on x-axis. Number of patients per month with respiratory sample tested positive for BoV, CoV, InV, MPV, RSV, PIV, or RV/EV.

2021. However, there was a slight increase of the RoV detection rate in January 2022, which temporally corresponded to the pre-pandemic seasonality of RoV detection.

Discussion

This study describes monthly rates of detection of viral pathogens in pediatric patients admitted to BCH before the COVID-19 pandemic as well as during the COVID-19 pandemic at different levels of public NPI measures.

Detection rates of non-seasonal viruses such as RV/EV in the respiratory tract and AdV in stool showed no change during the period of NPI measures and no immediate change after major NPI withdrawal. In contrast, detection rates of the seasonal viruses BoV, CoV, PIV, and RSV decreased during the period of NPI measures and showed an out of season increase after withdrawal of major NPI measures (Figure 1D). Likewise, rates of detection of the seasonal viruses InV, MPV, and RoV were diminished during the period of NPI measures. However, the detection rates of these viruses, as opposed to those of BoV, CoV, PIV, and RSV, stayed low after withdrawal of major NPI measures or returned to the pre-pandemic seasonality.

Previous studies showed that enveloped viruses such as InV, MPV, PIV, and RSV were detected at a lower frequency in children during the COVID-19 pandemic, whereas non-enveloped viruses such as Rhinoviruses were even detected at a higher frequency (5–7). This was attributed to non-enveloped viruses penetrating, whereas enveloped viruses not penetrating surgical masks (8). In our study we confirmed a stable rate of detection of RV/EV as non-enveloped viruses in children during the COVID-19 pandemic. BoV transmission might be more susceptible to NPI such as FFP2 masks than RV/EV transmission, although Bocaviruses share features with Rhinoviruses, such as the small size and the lack of a viral envelope (Table 1). The seasonal peak of the detection rate of BoV was blunted in winter 2020/21, in a period with an increased strictness of NPI measures in Berlin as a response to high incidence of SARS-CoV-2 infections. Overall, in this study the decline of virus detection frequency in children during the pandemic (April 2020 to May 2021) was related to respiratory viruses with previous seasonality of the detection frequency, regardless of their size or whether they bear a viral envelope.

Interestingly, we observed an increase of detection rates shortly after withdrawal of major NPI measures only for part of the airborne/droplet transmitted viruses. The detection rate of RSV in children rapidly increased several months before the pre-pandemic RSV season and, in October 2021, even exceeded all pre-pandemic detection rates. This caused a disease burden of pre-school children that possibly surpassed the burden of SARS-CoV-2 infections in this age group during the COVID-19 pandemic. The out of season increase of RSV detection is in line with studies from a number of countries covering five continents (9–14). Several studies also reported rates of infection by RSV that surpassed historical infection rates after public health measures had been reduced (15–17). An increased detection rate of PIV following a period of less frequent detection during the COVID-19 pandemic, as in our study, was described also for the US population (13). This increase occurred in May and June 2021, which corresponded to the pre-pandemic PIV season in the US. In our study we showed an increase of the PIV detection rate specifically for children. In contrast to the US, in our study the peaking PIV detection rate in summer 2021 was clearly delayed by 2 months, in relation the pre-pandemic PIV season. As in the US population, the seasonal peak of CoV detection was also delayed in our study in children by several months (13). To our knowledge, this is the first study that shows a rapid out of season increase of the detection rate of BoV after the withdrawal of major NPI measures.

There are a variety of mechanisms that may account for the out of season increase of detection rates of the described viruses after May 2021. RSV is proposed to interfere with other viruses at the population and host level, which might be a reason for the surge of the RSV infection rate in the absence of InV or Rhinoviruses (18, 19). Another possible explanation, however, is that the rise in the RSV infection rate might be caused by a lack of immunity of children due to missing contact to RSV during pregnancy and early childhood and waning of immunity in older children and the general population, leading to a so-called "immune-debt" (20–24). In other studies, an increasing burden of infection by RSV during the COVID-19 pandemic was not well correlated with school openings (22, 25). In our study, the increase in RSV frequency was temporally associated with several factors: opening of public buildings, schools, restaurants, night life and workplaces, and increasing tourism (Figure 1). On the contrary, the reopening of kindergartens preceded the peak of RSV infection frequency by more than 2 months. This observation is compatible with the thesis that adults and adolescents, not younger children, might be the major reservoir of RSV (21). The concept of an "immune-debt" might also explain the substantial increase in BoV and PIV detection frequency in children in our study after months of NPI measures. The detection rate of RSV during the COVID-19 pandemic was even lower than that of BoV and PIV, and the increase of BoV and PIV detection frequency preceded the one of RSV. Hence, RSV seems more susceptible to the mechanisms described above, including NPI measures. The frequency of InV and MPV detection was even lower than that of RSV during the pandemic and the delay of a surge in detection rates of InV and MPV was even longer than the delay of the current surge of RSV. The detection rate of MPV strongly adhered to a certain seasonality, both before and after the period of NPI measures. This is not well explained by concepts such as the immune debt or viral interference, but rather by changes in social life regarding winter season. Interestingly, in contrast to MPV, InV did not reappear. This might be attributable to the

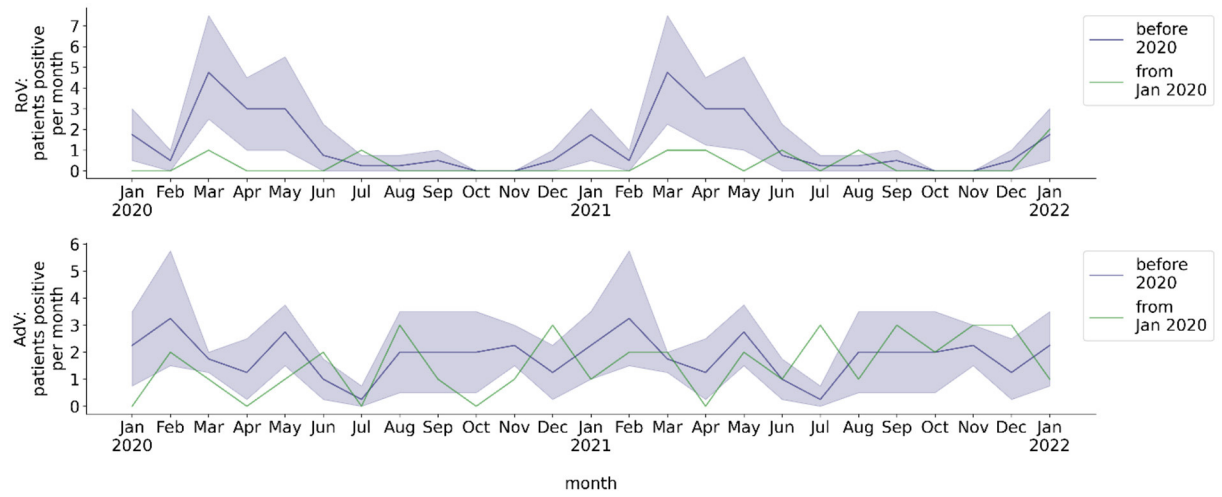

FIGURE 4
Monthly number of hospitalized patients at BCH positively tested for gastrointestinal viruses. Blue line: mean 2016-2019, blue shade: 95% confidence interval 2016-2019, green line: absolute number per month from 2020, calendar months 2016-2019 are shown as a historical reference and correspond to calendar months, not to year on x-axis. Number of patients per month with stool sample tested positive for RoV or AdV.

TABLE 1 Overview of viruses considered in this study.

Virus	Abbreviation in this article	Size (nm)	Enveloped
Adenoviruses	AdV	90–100	No
Human Bocavirus	BoV	20	No
Coronaviruses (229E, NL63, HKU1, OC43)	CoV	80–120	Yes
Influenzaviruses	InV	80–120	Yes
Human Metapneumovirus	MPV	150–600	Yes
Parainfluenzaviruses	PIV	150–250	Yes
Respiratory Syncytial viruses	RSV	120–300	Yes
Rhino-/Enteroviruses	RV/EV	20–30	No
Rotavirus	RoV	70	No

geographical globality of changes in social life and NPI measures. The reappearance of InV will have to be monitored carefully during the following months. In contrast to all other respiratory viruses, the RV/EV detection rate declined in July and surged in November 2021, exceeding pre-pandemic maximum monthly detection rates. Both the decline and the extreme surge thereafter might be a consequence of viral interference, as they were temporally associated with the increase of detection rates of BoV, RSV and PIV and their subsequent decrease.

A decline in the frequency of RoV detection in Germany during the COVID-19 pandemic was previously reported by the Robert Koch Institute, Berlin (26). Our study confirms this observation specifically for children. In contrast to RoV, however, there was no decrease of the rate of AdV detection in the stool of children with diarrhea, compared to the pre-pandemic phase. This might be attributable to AdV not solely relying on fecal-oral transmission and on children as the major reservoir but being transmitted via droplets and surfaces and involving an adult reservoir. In contrast to the respiratory viruses described above, we observed no increase of the RoV detection rate immediately following the withdrawal of major NPI measures. Due to ongoing vaccination against RoV during the COVID-19 pandemic, there might exist less of an "immune-debt," which might be a reason for the missing surge of the RoV detection rate.

From June 2021 to January 2022 we observed an increased monthly testing rate for respiratory viruses and Rotaviruses (Figures 2A,B). This was temporally associated with an increased detection rate of several respiratory viruses, but also with a subsequent decrease in the detection rate of several viruses. It reflected an increased burden of patients hospitalized due to respiratory and gastrointestinal infections since June 2021. Rather than being an explanation for increased virus detection rates, rising testing rates since June 2021 were a consequence of the increased rate of viral infections. At the beginning of the COVID-19 pandemic, we and other groups observed a decrease in monthly numbers of outpatient presentations (1, 27). Yet this cannot explain the changes in virus detection rates during the pandemic, as patients at BCH only undergo testing when admitted as inpatients to the hospital.

This study also has limitations. It is a single center study including a limited number of patients admitted to the hospital for inpatient care. Multi-center or multinational analyses may

prove whether the effects of NPI measures on the seasonality of virus infections we observed here, especially on the detection frequency of BoV and PIV, are transferable to other cities and hospitals. The study includes only the first 8 months after withdrawal of major NPI measures.

In summary, after months of NPI measures to prevent SARS-CoV-2 transmission during the COVID-19 pandemic, withdrawal of major measures led to a surge of infection rates of other viruses such as BoV and RSV in Germany. The infection rates of each of these viruses reached or even surpassed maximum pre-pandemic monthly infection rates. This caused a substantial disease burden in pre-school children. The peaking infection rates of these viruses might be explained by a lack of formation of immunity in infants and waning of immunity in older children and the general population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

NT, NU, VC, MM, and TK conceptualized the study. NT designed instruments for the data analyses, carried out the data analyses, and drafted the initial manuscript. NT, NU, AE, AK, LR, AG, HR, LM, HB, and TK carried out laboratory analyses and collected or provided data. NT, NU, AE, LM, HB, MM, and TK reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

Authors NU, AE, AK, and HB were employed by Labor Berlin GmbH, a joint subsidiary of the public hospitals Charité Universitätsmedizin Berlin and Vivantes — Netzwerk für Gesundheit GmbH.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Extracorporeal membrane oxygenation in the care of a preterm infant with COVID-19 infection: Case report

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Coronavirus disease 2019 (COVID-19) was first reported to the World Health Organization (WHO) in December 2019 and has since unleashed a global pandemic, with over 518 million cases as of May 10, 2022. Neonates represent a very small proportion of those patients. Among reported cases of neonates with symptomatic COVID-19 infection, the rates of hospitalization remain low. Most reported cases in infants and neonates are community acquired with mild symptoms, most commonly fever, rhinorrhea and cough. Very few require intensive care or invasive support for acute infection. We present a case of a 2-month-old former 26-week gestation infant with a birthweight of 915 grams and diagnoses of mild bronchopulmonary dysplasia and a small ventricular septal defect who developed acute respiratory decompensation due to COVID-19 infection. He required veno-arterial extracorporeal membrane oxygenation support for 23 days. Complications included liver and renal dysfunction and a head ultrasound notable for lentriculostriate vasculopathy, extra-axial space enlargement and patchy periventricular echogenicity. The patient was successfully decannulated to conventional mechanical ventilation with subsequent extubation to non-invasive respiratory support. He was discharged home at 6 months of age with supplemental oxygen *via* nasal cannula and gastrostomy tube feedings. He continues to receive outpatient developmental follow-up. To our knowledge, this is the first case report of a preterm infant during their initial hospitalization to survive ECMO for COVID-19.

KEYWORDS

COVID-19, ECMO, neonate, prematurity, case report

Background

Coronavirus disease 2019 (COVID-19) was first reported to the WHO in December 2019 and has since unleashed a global pandemic, with over 518 million cases reported by May 10, 2022 (1). Neonates represent a very small proportion of those patients, with few requiring invasive support for acute infection (2, 3). Among reported cases of neonates with symptomatic COVID-19 infection, the rates of hospitalization remain low (4). Most reported cases in infants and neonates are community acquired with mild symptoms, most commonly fever, rhinorrhea, and cough (4). Very few require intensive care, with one comprehensive study reporting 3% of pediatric patients, ages 1 day to 16 years. (5). We present a case of a 2-month-old former 26-week gestation neonate who required veno-arterial extracorporeal membrane oxygenation (ECMO) support for acute respiratory decompensation due to COVID-19 infection. To our knowledge, this is the first case report of a preterm infant during their initial hospitalization to survive ECMO for COVID-19.

Case

Our patient is a former 26-week gestation premature male infant with a birthweight of 915 grams and diagnoses of bronchopulmonary dysplasia and a small ventricular septal defect. He had a protracted NICU course notable for respiratory distress syndrome requiring surfactant therapy, intubation with mechanical ventilation for 6 days, and non-invasive positive pressure with through day of life (DOL) 52, and continued to require supplemental oxygen through DOL 68 and 36 weeks post-menstrual age, classifying his BPD as mild according to the current NICHD guidelines. Then, on DOL 82, he underwent an elective laparoscopic bilateral inguinal and umbilical hernia repair and circumcision. As per hospital policy, he had a negative COVID-19 screening test 2 days prior to this surgery. At this time, the patient weighed 2560 grams and had a stable supplemental oxygen requirement of 0.5 liters per minute FiO2 0.40 *via* nasal cannula. His parents visited regularly and were present on the day of surgery. The evening of his surgery, his mother began feeling ill and subsequently tested positive for COVID-19. The immediate post-operative course was uneventful.

On postoperative day 2 (DOL 84), the patient started with new onset severe apneic episodes requiring increasing respiratory support. An evaluation for infection was performed, including a complete blood count, blood and urine cultures and a comprehensive respiratory PCR panel that included COVID-19 testing. Due to worsening clinical status, he was started on broad spectrum antibiotics and given a blood transfusion for anemia. The comprehensive respiratory PCR panel resulted positive for COVID-19, while tests for other respiratory viral pathogens and bacterial cultures were negative. On DOL 86, 2 days after the diagnosis of COVID-19, the patient had a remarkably abrupt decompensation in his respiratory status resulting in hypoxic and hypercarbic respiratory failure. Chest radiograph revealed rapidly developing bilateral dense infiltrates. Aggressive medical management including conventional ventilation, high frequency oscillatory ventilation and inhaled nitric oxide (iNO) therapy failed. Due to continued hypoxia, hypercarbia and severe acidosis, and after consultation with pediatric surgery, he was placed on veno-arterial extracorporeal membrane oxygenation (ECMO) on DOL 87, 3 days after the diagnosis of COVID-19. Cannulas needed for veno-venous ECMO were not available due to product shortage at the time of this infant's cannulation, requiring the use of veno-arterial ECMO, using an 8 French arterial and a 10 French venous cannula due to the infant's size. Once on ECMO, the patient's hypoxia, hypercarbia and acidosis resolved with the pump flow set at 120–140 milliliters/kilogram/minute, and he was anti-coagulated according to our institution's protocol using a heparin coated circuit, continuous heparin and antithrombin III infusions. Prior to ECMO cannulation his head ultrasound (HUS) showed a brain normal for age with mild prominence of the extra-axial spaces, however, approximately 12 h after cannulation a repeat HUS showed a left grade two germinal matrix hemorrhage. At this time, the anti-coagulation therapy was adjusted by decreasing the heparin infusion by 25%, according to our institutions protocol, to further reduce the anti-thrombin Xa level and minimize bleeding risk. There was no extension of the intraventricular bleed on subsequent daily HUS. Systemic hypertension necessitated the use of multiple antihypertensive agents to maintain a mean arterial blood pressure (MAP) <60 mmHg while on ECMO. Throughout the ECMO course, the patient required full ECMO support due to poor lung compliance. We were unable to offer any COVID-19 specific medicinal therapies due to insufficient data at the time that it would change the course of disease in an infant. After 15 days on ECMO, the circuit was changed due to the escalating need for multiple blood products. Around this time, serial HUS noted lentriculostriate vasculopathy, extra-axial space enlargement and patchy periventricular echogenicity. With an extended time on ECMO complicated by progressive renal and liver dysfunction, concerning HUS findings and the re-emergence of the need for multiple blood products, the patient was decannulated after 23 days on ECMO on DOL 110. Prior to decannulation, a family meeting was held to discuss treatment options, and the family requested ongoing aggressive medical management. Despite having poor lung compliance and ongoing pulmonary hypertension at the time of decannulation, he survived with short term use of inhaled nitric oxide and intravenous sildenafil. Lung compliance improved over time allowing progressive wean of ventilator support, and renal and liver function also improved to normal values. On DOL 141, he was extubated to non-invasive positive pressure

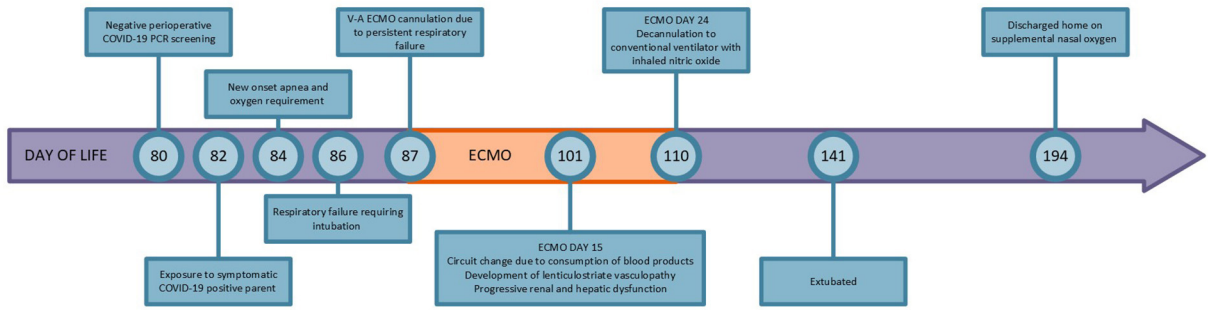

FIGURE 1
Timeline.

ventilation with a subsequent slow wean to a low flow nasal cannula. The ventricular septal defect was smaller consistent with expected spontaneous closure. Due to the inability to feed efficiently, a gastrostomy tube was placed on DOL 182. He was discharged home with supplemental oxygen *via* nasal cannula and gastrostomy tube feeds on DOL 194, weighing 5090 grams (see Figure 1). As an outpatient, he continues to receive treatment for his pulmonary hypertension and shows neurodevelopmental progress.

Discussion

With the onset of the COVID-19 pandemic, pediatric providers made clinical decisions based on limited information in attempt to reduce the risk of disease transmission to exposed infants and children and their providers. Initial guidance from the American Academy of Pediatrics was published online on April 2, 2020 and has been updated subsequently (6). While early disease impact was notable on pregnant women, increasing complexity of maternal care and at times leading to premature delivery, illness directly attributable to COVID-19 in infants was limited. Amongst children, multisystem inflammatory syndrome in children (MIS-C) was the greatest source of COVID-19 derived illness. With the onset of the Delta variant (7), an increasing number of pediatric cases of acute COVID-19 infection were reported, including increased severity of illness and death. Although the strain was not specifically identified, the timing of our patient's infection coincided with the Delta variant accounting for most cases in our region (8). As Delta and other novel variants un-fold in the COVID-19 pandemic, this case illustrates multiple challenges facing providers in the NICU and those caring for NICU graduates, in addition to direction for future research.

As with other respiratory viral infections, preterm infants infected with COVID-19 may present with apnea as an initial clinical finding. Early recognition of COVID-19 may expand options for therapies and reduce the likelihood of developing a severe disease course. As the treatment options for COVID-19 continue to evolve, increasing information about the efficacy of pharmacologic therapies such as monoclonal antibodies and antiviral therapies has become available for adults and older children. Complete information on the use of these products isn't available in children under age 12 (9). With consultation of pediatric infectious disease and immunology specialists, we treated with dexamethasone but did not offer any specific COVID-19 therapy to this infant. More research is needed on the use of specific COVID-19 treatments in the pediatric population, especially in younger children.

Prematurity with resultant lung disease in the form of mild bronchopulmonary dysplasia (BPD), and potentially the stress of surgery, placed our patient at increased risk for significant disease from COVID-19 (10). Other NICU patients and NICU graduates are likely at higher risk for symptomatic and severe COVID-19 infection. Efforts at disease prevention in this at-risk group should be paramount and include avoidance of sick contacts, COVID-19 vaccination of patient care providers, and when available, vaccination efforts for this susceptible population. Given the timing of the exposure and illness onset, it is likely our patient's COVID-19 infection was acquired from his mother while visiting him in the NICU, despite hospital screening procedures and universal masking for all visitors. Our patient's mother delivered prior to the third trimester where maternal vaccination may have provided some protection for the infant (11). The parents were not vaccinated which was not a requirement in our unit. However, our unit had visitors restricted to the two primary caregivers at this time with recommendations for mask wearing while in the room with their infant. In many NICUs across the country, varying degrees of visitor restrictions were enacted in attempts to limit nosocomial spread of COVID-19 to patients and health care workers (12). Balancing the necessity to protect our at-risk population while promoting infant-caretaker bonding and neurologic development continues to pose a challenge. This case highlights one side of this equilibrium, yet the impact of COVID-19 on the developmental outcomes of NICU patients

may never be fully understood. Further development of methods to promote bonding and development and engaging in family centered care while limiting exposure is needed.

The developmental outcome for our patient remains uncertain, and appropriate developmental follow-up and interventions are ongoing. Our patient did develop a left grade 2 germinal matrix hemorrhage after initiation of ECMO that resolved and had subsequent findings of lenticulostriate vasculopathy. The etiology and consequence of lenticulostriate vasculopathy in this case is uncertain as it is reported to occur in 15% of preterm infants and is not independently associated with adverse neurodevelopmental outcomes (13). While COVID-19 infection is associated with a higher risk of thrombotic events in children and adults, the impact of COVID-19 infection on coagulopathy in infants is uncertain (14).

Although the literature is scarce regarding the use of ECMO support for COVID-19 pediatric and neonatal patients, the ELSO guidelines support its use if indicated, with decision to cannulate based upon "existing indications and thresholds per currently published guidelines" (15). Since the patient carried a diagnosis of Mild BPD, was in a relatively healthy state with low O2 requirements nearing discharge, with reasonable chance of recovery, we chose to place the patient on ECMO. At the time of this report, there have only been a few cases reported of neonates who were supported on ECMO for COVID-19. Both reported cases involved neonates with congenital heart disease, one pre- and the other post-op with only one of those neonates surviving to discharge (16, 17). Compared to other published case reports, our patient had a more rapid progression from diagnosis to intubation and need for ECMO support, and he also had a longer duration of ECMO (18). We suspect that the comorbidities of prematurity and BPD may have impacted the rapidity of our patient's decline and prolonged need for ECMO support, likely increasing his risk for morbidity and mortality.

To our knowledge, this is the first case report of a preterm infant during their initial hospitalization to survive ECMO for COVID-19. Our rare case serves as a reference point for neonatologists as this pandemic continues to affect our unique patient population.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JP-E, CM, and EM gathered the patient data, drafted the initial manuscript, and reviewed and revised the manuscript. JL aided in drafting the initial manuscript and reviewed and revised the manuscript. BB and DY reviewed the manuscript for intellectual content and reviewed and revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hematologic manifestations of coronavirus disease 2019 in children: Case-series report and a review

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On 11 March 2020, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) was declared as a pandemic by the World Health Organization (WHO). As the COVID-19 pandemic has ravaged worldwide, children have not been unaffected. Information gleaned from adult experience with the disease has aided in disease detection and treatment strategies in children. Numerous cases have been described in adult literature about hematologic manifestations of COVID-19. This case series aims to report several hematologic presentations in patients with COVID-19 and multisystem inflammatory syndrome in children (MIS-C, an immune-mediated reaction leading to severe COVID-19 illness) with and without a primary hematologic disorder.

KEYWORDS

COVID-19, children, hematologic, manifestations, case series

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), was announced as a pandemic by the World Health Organization (WHO) on 11 March 2020. Since then, the COVID-19 pandemic has significantly impacted healthcare systems, socioeconomic aspects, and livelihoods (1–5). COVID-19 has had overwhelming morbidity and mortality in the adult population. However, the severity of COVID-19 in the pediatric population has also been reported (6, 7). COVID-19, though primarily respiratory in origin, results in a multisystemic disorder that involves the cardiovascular, respiratory, gastrointestinal (GI), neurologic, hematopoietic, and immunologic systems (8). Of particular interest in this case series are the hematopoietic sequelae of COVID-19, which manifest themselves as laboratory abnormalities and coagulopathic and thrombotic events. Hematologic changes occurring early in COVID-19 include lymphocytopenia and thrombocytopenia, while the late-stage changes include more severe thrombocytopenia, neutrophil elevation, and severe coagulation disorder (9). We reported several pediatric case presentations and detailed hematologic findings.

Case description and diagnostic assessments

Case 1: Multisystem inflammatory syndrome in children with severe refractory thrombocytopenia

An otherwise healthy 4-year-old girl with a history of SARS-CoV-2 infection for 4 weeks before the presentation was admitted, followed by 4 days of sore throat, fatigue, and decreased oral intake. They had tested positive for COVID-19 on December 9, 2021 (2 months before presentation) and were symptomatic for approximately 1 week while quarantined at home. The patient was admitted to the pediatric floor with a suspected diagnosis of COVID-19 or multisystem inflammatory syndrome in children (MIS-C). The rheumatology service was then consulted. Initial laboratory results showed mild anemia (Hb, 10.9 mg/dl), neutrophilia (75%), lymphopenia (11%), pandemic (12%), elevated inflammatory markers (erythrocyte sedimentation rate (ESR), 100 mm/h; C-reactive protein (CRP), 23.6 mg/dl; ferritin, 350 ng/ml; procalcitonin, 46.25 ng/ml), hyponatremia (135 mmol/L), markedly elevated D-dimer (1,398 ng/ml), elevated B-type Natriuretic peptide test (BNP) (179 pg/ml), normal troponin I $<$ 0.030 ng/ml, transaminitis (alanine aminotransferase (ALT), 57 IU/L and AST, 97 IU/L), and normal electrocardiogram (EKG). Due to suspected MIS-C, rheumatology recommended two doses of 1 g/kg intravenous immunoglobulin (IVIG) at 24 h interval. The patient later developed hypotension and persistent tachycardia refractory to resuscitation with crystalloid; she was started on 30 mg/kg/day methylprednisolone and transferred to the Pediatric Intensive Care Unit.

The patient required treatment with vasopressors due to worsening hypotension. Prophylactic anticoagulation with Lovenox was initiated. The hospital course was further complicated by acute hypoxic respiratory failure requiring intubation. Clinical evaluation supported a diagnosis of MISC-C as per CDC guidelines. Inflammatory markers were markedly elevated, necessitating treatment with anakinra at 2 mg/kg, and later increased to a maximum dose of 6 mg/kg. Due to abdominal distension and firmness to palpation, ultrasound (US) and CT scans were obtained and showed signs concerning acalculous cholecystitis. Severe third spacing, electrolyte imbalances (potassium, phosphorus, and sodium), and hypoalbuminemia occurred. Gastroenterology and surgery teams evaluated the patient and recommended a conservative approach (non-surgical approach). Electrolyte replacements and albumin infusions were administered. Echocardiograms obtained showed relatively normal cardiac muscle function. In the following days, a complete blood count (CBC) revealed a significant drop in hemoglobin (nadir of 6.6 mg/dl), which required blood transfusions on two separate occasions, and severe thrombocytopenia (bottom of 23 K/μl). Workup for thrombotic thrombocytopenic purpura and disseminated intravascular coagulation was negative. Literature search assured that this was post-MIS-C immune thrombocytopenia observed while patients are recovering from MIC-S, and treatments known for treating ITP are sufficient. Two doses of IVIG were given, after which platelets increased slightly; subsequently, two doses of romiplostim were administered, after which platelet count increased substantially. As the patient improved clinically, inflammatory markers down trended, and lymphopenia and thrombocytopenia resolved. The patient was transferred to the Pediatric Ward for methylprednisolone, anakinra wean, physical therapy, and nutritional optimization. She was discharged home on hospital day 21.

Case 2: Sickle cell disease with fever and vaso-occlusive pain crises

A 15-year-old girl with a history of sickle cell disease (SCD)-HbSC genotype, on hydroxyurea and voxelotor, presented to the Emergency Department (ED) with a 3-day history of fever, chest, abdomen, and lower extremity pain that failed to respond to pain medications. On presentation to the ED, she was afebrile and stable on room air (RA) without signs of respiratory distress. Initial laboratory results showed hemoglobin of 9.8 g/dl (baseline, 11 g/dl), lymphopenia, and elevated reticulocyte count to 3.5%; SARS-CoV-2 PCR was positive, and chest X-ray was negative for acute chest syndrome. She was admitted to the inpatient unit to manage a vaso-occlusive pain crisis. Pain control was started with a hydromorphone patient-controlled analgesia (PCA) pump and scheduled IV ketorolac. She remained afebrile and stable on RA. Her pain was adequately controlled, which allowed her to later transition to oral oxycodone and ibuprofen. She was discharged home on day 3 of hospitalization.

Case 3: Sickle cell disease with fever and vaso-occlusive pain crises

A 16-month-old boy with a history of SCD-HbSC genotype, on twice-daily amoxicillin prophylaxis, presented to the ED with 4 days of cough, vomiting, one day of fever, and questionable limping. He was afebrile and stable on RA without signs of respiratory distress. Initial laboratory results showed hemoglobin of 10.6 g/dl (baseline, 11.5 g/dl), elevated reticulocyte count to 3%, and average differential count; SARS-CoV-2 PCR was positive, and chest X-ray was negative for acute chest syndrome. Liver function tests and basic metabolic panel were within the normal ranges. He was admitted to the Pediatric Ward to manage vaso-occlusive pain crisis and rule out sepsis. The pain was adequately controlled with morphine

and acetaminophen. He remained afebrile and stable on RA; blood culture showed no growth for the past 48 h. The pain was adequately controlled, which allowed the transition to oral oxycodone and ibuprofen. Hemoglobin remained stable with a decrease in reticulocyte count. He was discharged home on day 3 of hospitalization.

Case 4: Hereditary spherocytosis with acute on the chronic hemolysis

A 12-year-old boy with a history of hereditary spherocytosis and previously vaccinated against SARS-CoV-2 presented to Emergency Care with a 2-day-history of cough, congestion, abdominal pain, nausea, and vomiting. On examination, he was afebrile, stable on RA, and had significant icteric sclerae without hepato-splenomegaly or abdominal tenderness. Laboratory results showed slight decrease in red blood cell count (3.66 M/μl vs. 3.81 M/μl), hemoglobin (11 g/dl vs. 10.8 g/dl), hematocrit (29.3% vs. 29.7%), and mean corpuscular hemoglobin concentration (36.7 g/dl vs. 37 g/dl) compared to his baseline. Liver function tests showed elevated total bilirubin (3.2 mg/dl vs. 2.1 mg/dl) compared to baseline; gamma-glutamyl transpeptidase and lipase were within normal ranges, as CRP and ESR. Given the patient clinical stability, he was discharged home and under close monitoring for ongoing hemolysis. A repeat blood count obtained 48 h later showed a further drop in hemoglobin counts at 8.6 g/dl, and reticulocyte count was elevated at 13.6%. Hemoglobin returned to a baseline count of 11.3 g/dl after 1 week.

Case 5: Post-coronavirus disease 2019 immune thrombocytopenia

A 13-month-old boy with a recent history of COVID-19 infection (SARS-CoV-2 PCR positive) 2 weeks before presented to Emergency Care for worsening petechiae over a 3-day course. The petechiae started on his stomach and chest, spreading to his extremities later. There were no previous episodes of mucosal bleeding or epistaxis, although parents described multiple bruises on the legs, some of which were attributed to bumps or falls. The initial laboratory results showed a platelet count of 10 K/μl, few burr cells on the peripheral smear, mild elevation of coagulation tests (PTT 44 s, PT 13.5 s, INR 1.22), normal fibrinogen (258 mg/dl), elevated Factor V (289%), slightly low Factor VII (48%) and normal Factor X (113%), elevated D-dimer (685 ng/ml), and normal inflammatory markers (ESR, 16 mm/h; CRP $<$ 0.5 mg/dl; ferritin, 37.3 ng/ml). He was admitted to the inpatient unit for the management of thrombocytopenia, likely secondary to the recent COVID-19 illness. Treatment was initiated with 1 g/kg IVIG and 1 mg/kg methylprednisolone. Repeat CBC showed an increase of platelet count initially to 14 and 40 K/μl at 20- and 35-h post-treatment, respectively. He was discharged home and repeat platelet count checks achieved normal ranges within several weeks of hospital discharge, and platelet counts have remained stable.

Discussion

Entry of the SARS-CoV-2 to human host cells requires the cellular receptor angiotensin-converting enzyme 2 (ACE) and serine protease TMPRSS2 for spike protein priming. The virus enters the target cells *via* interaction between viral surface S spike protein and ACE2 (type 1 integral membrane receptor) expressed in multiple tissues, i.e., lung, heart, kidney, GI, and vasculature (6, 10). Cleavage of the viral S protein is done by TMPRSS2, following which the viral RNA genome is released, and the viral replication cycle starts (6). After entry, the virus can multiply and disseminate into the airway after shutting down the IFN type 1 antiviral pathway and spreading from the lung to other ACE2-expressing tissues (6). The direct cytotoxic effect of the virus on endothelial cells and microvascular occur, followed by inflammation and excessive release of cytokines which further aids the development of a prothrombotic state (6).

Coronavirus disease 2019 is primarily a respiratory illness that ranges from asymptomatic infection to a more severe form of the disease that is multisystemic. Most children who develop COVID-19 have mild symptoms or remain asymptomatic. Numerous studies have reported children with certain underlying medical conditions, such as chronic respiratory illness, moderate-to-severe asthma, obesity, diabetes, sickle cell disease, and cancer (11). COVID-19 causes a variety of hematologic findings that have been reported in the literature. Changes in peripheral blood count, including lymphopenia, thrombocytopenia, and elevated D-dimer levels, are among the most reported results (12). ACE is expressed on lymphocytes, and the virus utilizes this to cause a direct cytotoxic effect leading to lymphopenia, ACE2 receptors in hematopoietic stem cells in BM-pancytopenia (depletes all forms of blood cells), and finally, TNF-alpha induction during the cytokine storm mediates cell apoptosis (13). Neutrophilia is preceded by a cytokine storm, superimposed bacterial infection (13). Thrombocytopenia results from the destruction of hematopoietic precursors in the marrow, destruction by virus-induced autoantibodies which form immune complexes and are cleared from the body, the consumption of platelets during the coagulation cascade, and thrombi formation (13).

The standard CBC changes from COVID reported in children include lymphopenia, leukocytosis/leukopenia, thrombocytopenia, and neutrophilia (14).

Other significant lab findings include elevation in inflammatory markers, i.e., CRP, ESR, and procalcitonin (14). A study pooled 24 studies involving 624 pediatric cases with lab-confirmed COVID-19, reporting data on 27

biomarkers (0–17.5 years, female 43%) (15). Only 6 of the 24 studies addressed lab findings in patients with severe COVID-19 illness. Due to limited data and high variation across these studies, it could not be analytically pooled. Observations revealed that a few patients had elevated WBC, and increased and decreased lymphocyte counts were observed at an equal frequency (18.4%). In addition, elevated D-dimer and PT could be seen (15). Lymphopenia, thrombocytopenia, and neutrophilia have all been used as hematologic biomarkers for prognosticating disease severity in adults (1). However, there are subtle differences in the pediatric population as lymphopenia is not observed as frequently in the pediatric population compared to the adult population, although the presence of lymphopenia can correlate with disease severity in the pediatric population (16). According to Per Cui et al., the reduction of lymphocytes in children was only 16%, but in adults, it was up to 43.1, 57.4, and 56%, respectively, reported by a previous meta-analysis; the reason for these differences may be related to the immune response of different organisms to novel coronavirus (17). Other abnormal lab parameters reported have included coagulation profile abnormalities (18).

In summary, hematologic parameters that are prognostic markers in COVID-19 have not yet been accurately identified in children. Although lymphopenia correlates with increased disease mortality in adults and other biomarkers, including CRP, procalcitonin (PCT), and ferritin, cytokine levels have been associated; these associations have not been reliably tested in children (15). It may be possible that the relative immaturity of the immune system in young children accounts for differences in viral susceptibility or response to infection, possibly explaining the differences in laboratory trends seen in the pediatric versus the adult population of patients with COVID-19 (15). Children with severe COVID-19 showed somewhat consistent trends of elevated lactate dehydrogenase (LDH), CRP, and PCT levels, as reported in adult patients with COVID-19 (16, 20). In addition, elevated D-dimer and PT trends were noted in children with severe COVID-19, although these variables have not been consistently measured across studies (15, 19). Nonetheless, these also overlap with findings reported in adult patients, which have been suggested as markers of the recently highlighted hypercoagulability status seen in patients with severe disease (15, 20). It is worth mentioning that further information on other biomarkers that may help in COVID-19 prognostication, such as IL-6 and serum ferritin levels, remains limited in children, as only two studies reported data on IL-6 in COVID-19, with none reporting data on serum ferritin; IL-6 was only elevated in 37.5% of severe pediatric cases (15). Additional studies should, therefore, incorporate the measurements of IL-6, given that this biomarker is commonly elevated in viral respiratory tract infections and perhaps plays a vital role in the cytokine storm seen with the disease, which may make it part of a risk stratification test [16,22]. Morphologic changes in blood cells have also been reported. Neutrophils show clumped chromatin with toxic granulation and vacuolization, pseudo Pelger–Huet deformity, and bilobed nuclei seen in acute infections (21). Lymphocytes present with abundant pale to dark blue cytoplasm with lymphoplasmacytic features b, and basophilic stippling is seen in red blood cells (21). Platelet clumping has been observed and activated macrophages with abnormal shapes and cytoplasmic vacuolization were also present (21). COVID-19-associated coagulopathy, a distinct form of coagulopathy, has been reported in COVID-19 infection. The pathophysiology involves SARS-CoV-2 entering cells by binding to the angiotensin-converting enzyme 2 receptors; a similar cascade was described earlier. The virus also targets the endothelial cells, which widely express ACE-2 (22). Unchecked viral replication induces a florid host response characterized by dysregulation of inflammation and coagulation. Due to this inflammation and upregulated immune response, platelets are activated, and natural mechanisms of anti-coagulant are downregulated (22, 23). The dysfunction of the endothelium, another risk factor for coagulopathy, may lead to systemic damage with abnormal coagulation, kidney disorders, pulmonary embolism, and sepsis (22, 24). Other pathogenic mechanisms, including increased secretion of von-Willebrand factor (vWF) from damaged endothelium, TLRs, and complement activation, are involved in COVID-19-associated coagulopathy (22). Abnormal hematologic findings related to Covid-19 associated coagulopathy (CAC) include thrombocytopenia, prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT), and increased fibrin degradation product (FDP) levels, and elevated D-dimer has been associated with poor prognosis and an increased rate of mortality in patients with COVID-19 (22, 25). The most prevalent thrombotic complications of COVID-19 in children parallel those seen in adults and include deep vein thrombosis, pulmonary emboli, chest thrombi, and neurological thrombosis (26). A study reported the incidence of thrombotic events in hospitalized children with COVID-19 as 2.1 and 6.5% in those with MIS-C compared with 0.7% in those with asymptomatic COVID-19 infection (27). Cancer, central venous catheter, older age, and MIS-C are risk factors for thrombosis in children and adolescents with COVID-19 or MIS-C. Mortality was high (28%) in children and adolescents with MIS-C or COVID-19 who developed thrombosis (26). No high-quality evidence demonstrates the safety and efficacy of therapeutic over prophylactic dosing in children. The COVID-19 Anticoagulation in Children Thromboprophylaxis (COVAC-TP) Trial aims to evaluate the safety, dose requirements, and exploratory efficacy of twice-daily subcutaneous enoxaparin as venous thromboembolism (VTE) prophylaxis in children (birth to 18 years) hospitalized with signs and symptoms of SARS-CoV-2 infection (i.e., COVID-19) was launched in June 2020 (27).

Consensus-based clinical recommendations and research priorities for anticoagulant thromboprophylaxis in children hospitalized for COVID-19-related illness (ISTH) have given broad recommendations for AC prophylaxis (28). Among these

recommendations include anticoagulant thromboprophylaxis (in combination with mechanical thromboprophylaxis with sequential compression devices where feasible) should be administered in children hospitalized with COVID-19-related illness (e.g., MIS-C) who have superimposed clinical risk factors for hospital-associated VTE or markedly elevated plasma D-dimer levels (e.g., ≥5 times the upper limit of typical values), in the absence of contraindications *[expert opinion, with strong consensus (83%, 15/18)]* (29).

Low molecular weight heparin (LMWH) or unfractionated heparin (UFH) is the anticoagulant of choice in children with acute infection. It is commonly used over UFH due to its reliable pharmacokinetics, pharmacodynamic responses, and longer half-life (28). LMWHs may have additional benefits due to their anti-inflammatory and immunomodulatory properties, i.e., potential antiviral property as it interacts with the SARS-CoV-2 spike S1 protein receptor-binding domain and interferes with its engagement with the receptor (28). Opinions generally differ on monitoring anti-Xa for prophylaxis dosing (28, 29). Higher doses of LMWH/UFH may be needed due to acquired heparin resistance and deficiency of antithrombin as the disease progresses (28).

Bleeding events occur less frequently than thrombotic events and are incompletely understood (18). Microvascular thrombosis and thrombocytopenia are reported to contribute to bleeding events in pediatric patients; cutaneous bleeding, presenting as petechiae, microhemorrhages in the extremities, erythema, macular eruptions, and purpura, often labeled as chilblains was the most common bleeding event reported (18).

In disease-specific hematologic conditions, children with sickle cell infected with COVID-19 were reported to be asymptomatic (25.5%) or to experience mild-to-moderate symptoms (65.6%), while some others had more severe symptoms (8.2%) (30). This cohort was associated with a 40% hospitalization rate, 5.8% ICU admission, 1.1% ventilator use, and 1% death (0.3%) (30, 31). The reported risk factors for worse outcomes included a history of pain and heart/lung comorbidities (30, 31). Our reported sickle cell patients presented with moderate symptoms requiring hospitalization and recovered without additional intensive unit requirements.

Thrombocytopenia is caused by the direct effect of SARS-CoV-2 on platelet production, autoimmune destruction of platelets, or increased platelet consumption (microthrombi formation) (1). A meta-analysis study reported significant thrombocytopenia in patients with more severe than mild diseases (1).

The reported patient with MIS-C developed severe thrombocytopenia, which was deemed multifactorial, and eventually responded to thrombopoietin receptor agonist (TPO-RA), which has since been discontinued due to sustained stable counts. Post-COVID-19 ITP and TTP (potential immune-mediated platelet destruction) have been reported; the likely mechanism is molecular mimicry between the antigens of SARS-CoV-2 and platelet glycoproteins (1). Our young patient developed post-COVID ITP, responded to treatment with IVIG, and continues to do well.

Severe anemia requiring transfusion was not commonly reported in pediatric COVID-19 presentations. However, SARS-CoV-2 could trigger hemolytic anemia (autoimmune) and lead to hemolytic crises in congenital hemolytic anemias (32). Hereditary spherocytosis is inherited hemolytic anemia caused by a genetic mutation that encodes the red cell membrane, resulting in red blood cells having an abnormal, spherical shape with decreased flexibility. Infections/illnesses can exacerbate hemolysis. In a previously reported case, a 4-year-old boy with a history of moderate hereditary spherocytosis (HS) and sickle cell trait without prior splenectomy presented with COVID-19 disease; experienced exacerbated hemolysis requires blood transfusions (33). In our patient vaccinated against COVID-19, hemolysis was mild, and packed red blood cells (PRBCs) transfusion was not needed.

It is important to emphasize that just like any other acute infection/illness, COVID-19 disease can worsen pre-existing or chronic hematologic diseases. For instance, in major patients with thalassemia who are splenectomized, when infected by SARS-CoV-2, these patients may also develop secondary bacterial infections.

As the pandemic continues to wax and wane, efforts need to be intensified to have a comprehensive register explicitly designed for the pediatric population to gather relevant information on the disease presentation, affectation, and clinical course. This will aid the formulation of appropriate treatment guidelines, as well as highlight the areas of focus that need to be improved.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

GO and JA: collection of data, review and reporting of data, and discussion section. AB: review of data and discussion section. All authors listed have made a substantial, direct,

and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.935236/full#supplementary-material

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Anakinra treatment in multisystemic inflammatory syndrome in children (MIS-C) associated with COVID-19

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Objective: The study aimed to report the efficacy and safety of anakinra treatment in patients with the refractory multisystemic inflammatory syndrome in children (MIS-C).

Methods: This is a cross-sectional retrospective study consisting of pediatric patients diagnosed with MIS-C who were treated with anakinra.

Results: Among the 378 patients diagnosed with MIS-C, 82 patients (21.6%) who were treated with anakinra were included in the study. The median age of patients was 115 (6-214) months. The median duration of hospitalization was 15 (6-42) days. Sixty patients (73.1%) were admitted to the pediatric intensive care unit. Patients were treated with a median dose of 2.7 mg/kg/day anakinra concomitant with IVIG and steroids. Intravenous anakinra was applied to 12 patients while 70 patients received it subcutaneously. Twenty-eight patients required high dose (4–10 mg/kg/day) anakinra. The median day of anakinra initiation was 2 (1-14) days and the median duration of anakinra use was 7 (1-41) days. No injection site reactions were observed while elevated transaminase levels were detected in 13 patients. Seventy-three patients (89.1%) were discharged without any sequela or morbidity. Seven patients (1.8%) died. Abnormal echocardiographic findings continued in two patients (2.4%) (coronary artery dilatation in one, low ejection fraction in one) at discharge and became normal on the 2nd month.

Conclusion: Based on the results of the study, anakinra was associated with clinical improvements and was safe for most patients with refractory MIS-C.

KEYWORDS

anakinra, COVID-19, multisystem inflammatory syndrome in children (MIS-C), treatment, refractory MIS-C

Introduction

Multisystemic inflammatory syndrome in children (MIS-C) is a hyperinflammatory condition that has recently entered our lives after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and presents with Kawasaki-like disease and/or shock-like findings. The first 8 cases were reported from England (1). Subsequently, similar case reports began to come from all over the world.

A total of 6,851 MIS-C cases have been reported and 59 deaths have been observed since May 2020 (2). Most of the patients were negative for RT-PCR and positive for SARS-COV 2 antibodies. Patients present with various clinical findings including fever, rash, conjunctivitis, gastrointestinal (GI) symptoms, coagulopathy, cardiac involvement, and shock-like findings. As distinct from Kawasaki disease (KD), left ventricular dysfunction is the predominant cardiac feature in patients with MIS-C. Laboratory findings revealed an increased inflammatory response and some of the patients may progress to macrophage activation syndrome (MAS).

The underlying pathogenesis has not yet been fully elucidated and an abnormal immune response is blamed as the main factor in the pathogenesis of MIS-C. Various cytokines are thought to cause hyperinflammation, including, interleukin-1 (IL-1), interferon-gama (IFNγ), IL-18, IL-6, IL-8, and IL-10 (3, 4). While intravenous immunoglobulin (IVIG) and corticosteroids are used in the first line of treatment, anakinra is recommended as a therapeutic option in resistant cases (5). Anakinra is a recombinant IL-1 receptor antagonist. It acts as an anti-cytokine by inhibiting the binding of both IL-1-α and IL-1-β to IL-1 receptors. Anakinra is used to mitigate organ damage by hindering the cytokine storm that occurs in MIS-C. In this multicenter study, we aimed to present the data of patients using anakinra for refractory MIS-C.

Methods

This is an international, cross-sectional study involving pediatric patients who were diagnosed with MIS-C and were treated with anakinra between May 2020 and December 2021, from six pediatric rheumatology centers Among the 378 patients diagnosed with MIS-C, 82 patients (21.6%) who were treated with anakinra were included in the study. The diagnosis of MIS-C was made according to WHO or CDC criteria (6, 7) and American College of Rheumatology Clinical Guidance (5) was used to guide the treatment. Anakinra dose has been adjusted according to the treating physician's decision. Clinical and laboratory findings, treatments, and outcomes were recorded retrospectively from medical charts and electronic files of the patients. Adverse events were defined as any undesirable or suspected reaction that occurred after anakinra treatment. Adverse events that prolonged hospital stay, or led to life-threatening conditions have been described as serious adverse events. Liver enzymes were accepted as increased if the elevation is ≥2 times the upper limit of normal. The definition of complete and incomplete KD was made according to American Heart Association (AHA) (8). The 2016 EULAR/ACR/PRINTO Classification Criteria for Macrophage Activation Syndrome was used for the classification of MAS patients (9).

Patients who had confusion, hypotensive course, sustained fever, hypoxia, multiple organ failures, and needed plasmapheresis, mechanical ventilation or ECMO were followed up in the intensive care unit. The study protocol was reviewed and approved by the Ethics Committee of the University of Health Sciences, Umraniye Training and Research Hospital (Approval No: B.10.1.TKH.4.34.H.GP.0.01/13) with the ethical principles laid down in the Declaration of Helsinki.

Statistical analyses

The statistical analyses were made by using SPSS version 21.0 (SPSS, Inc., Chicago, Illinois). The variables were investigated using visual (histogram, probability plots) and analytic methods (Kolmogorov Smirnov/Shapiro–Wilk's test) to determine whether or not they are normally distributed. Descriptive analysis was presented using proportions, mean, standard deviation (SD), median, minimum (min), and maximum (max) values as appropriate.

Results

Baseline characteristics of patients

The study group consists of 82 patients with refractory MIS-C. Among them, 48 (58.5%) were male and 34 (41.5%) were female. The median age of patients was 115 (6–214) months. Sixty (73.2%) patients had a history of close contact with a symptomatic COVID-19 patient. Nasopharynx SARS-CoV-2 PCR was positive in 4 patients (4.9%) and the SARS-CoV-2 antibody test was positive in 78 patients (95.1%).

The most common clinical finding was fever, followed by cardiac, GI, and mucocutaneous features (Table 1). The median duration of fever before the diagnosis was 5 (1–30) days. Of these 82 patients, 41 (50%) had a Kawasaki-like phenotype and 34 (41.5%) fulfilled the classification of MAS.

On the laboratory evaluation, lymphopenia was detected in 63 (76.8%) and thrombocytopenia in 33 (40.2%) patients. The C-reactive protein (CRP) levels were elevated in all patients while increased levels of erythrocyte sedimentation rate (ESR), ferritin, and procalcitonin were detected in 74 (90.2%), 43 (52.4%), and 58 (70.7%) patients, respectively. Five patients

TABLE 1 Clinical characteristics of MIS-C patients.

	Patients ($n = 82$)
Fever, *n* (%)	82 (100)
Mucocutaneous features	
. Polymorphous rash, *n* (%)	58 (70.7)
. Conjunctivitis, *n* (%)	51 (62.2)
. Oral changes, *n* (%)	29 (35.4)
. Extremity changes, *n* (%)	15 (18.3)
Cervical lymphadenopathy, *n* (%)	26 (31.7)
Mesenteric lymphadenopathy, *n* (%)	10 (12.2)
Hepatomegaly, *n* (%)	12 (14.6)
Splenomegaly, *n* (%)	10 (12.2)
Musculoskeletal features	
. Myalgia, *n* (%)	30 (36.6)
. Arthralgia, *n* (%)	28 (34.1)
Respiratory findings	
. Cough, *n* (%)	10 (12.2)
. Dyspnea, *n* (%)	17 (20.7)
Gastrointestinal findings	
. Abdominal pain, *n* (%)	52 (63.4)
. Nausea and vomiting, *n* (%)	28 (34.1)
. Peritonitis, *n* (%)	16 (19.5)
. Diarrhea, *n* (%)	21 (25.6)
. Bloody diarrhea, *n* (%)	5 (6.1)
Cardiac involvement	
. Hypotension, *n* (%)	56 (68.3)
. Tachycardia, *n* (%)	39 (47.6)
. Bradycardia, *n* (%)	8 (9.8)
. LV dysfunction or myocarditis, *n* (%)	34 (41.5)
. Mitral valve, *n* (%)	32 (39)
. Pericarditis, *n* (%)	14 (17.1)
. Coronary artery involvement, *n* (%)	4 (4.9)
Renal involvement, *n* (%)	3 (3.6)
Neurologic involvement	
. Headache, *n* (%)	18 (22)
. Loss of consciousness, *n* (%)	7 (8.5)

(6.1%) had elevated troponin-I levels, increased levels of pro-brain natriuretic peptide (BNP) were detected in 44 patients (53.6%), and D-dimer was increased in 69 patients (84.1%). Hypoalbuminemia and hyponatremia were detected in 59 (72%) and 55 (67.1%) patients, respectively (Table 2).

Treatments

Sixty (73.1%) patients required intensive care support within a median of 1.5 (1–15) days after hospitalization. The median duration of hospitalization was 15 (6–42) days and the median duration of stay in the intensive care unit was 5 (1–27) days. All patients received IVIG and corticosteroids. Thirty-one patients (31.8%) received pulsed methylprednisolone for three consecutive days (15–30 mg/kg/day; maximum dose: 1,000 mg/day) and then continued with a dosage of 2 mg/kg/day. The remaining 51 patients (68.2%) received 2 mg/kg daily dose of corticosteroids. In 12 patients (14.6%) IVIG treatment was completed to 2 g/kg in split doses. Twelve patients (14.6%) received intravenous and 70 patients (85.4%) received subcutaneous anakinra. Patients were treated with a median dose of 2.7 (2–10) mg/kg/day anakinra while the anakinra dose was increased to 4–10 mg/kg/day in 28 patients (34.1%). The median day of anakinra initiation was 2 (1–14) days and the median duration of anakinra use was 7 (1–41) days. Thirty-four patients (41.4%) received inotropic agents, and 19 patients (23.2%) received plasmapheresis. Prophylactic low-molecular-weight heparin (LMWH) (1 mg/kg/day) was given to 73 patients (89%).

TABLE 2 Laboratory results of the patients' at the initiation of anakinra treatment.

Complete blood count	
WBC, mm^3	9,530 (1,680–51,530)
Lymphocyte, mm^3	875 (160–9,410)
NLR	8.5 (0.9–72)
Hemoglobin, g/dL	10.8 (3.1–14.7)
Platelet, mm^3	170,500 (65,000–370,000)
Inflammatory markers	
CRP, mg/dL	16.1 (5.1–38.6)
ESR, mm/hr	47 (2–140)
Procalcitonin, ng/mL	11.3 (0.08–100)
Ferritin, ug/L	602 (127–13,437)
IL-6, pg/mL	390 (2–2,330)
Cardiac markers	
NT-pro-BNP, ng/L	5,550 (39–35,000)
Troponin-I, ng/L	0.01 (0–89.7)
Coagulation parameters	
D-dimer, μg/mL	3.95 (0.6–26.9)
Fibrinogen, g/L	543 (130–1,096)

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; NLR, neutrophil-lymphocyte ratio; NT-pro-BNP, N-terminal prohormone brain natriuretic peptide; WBC, White blood count.

Outcome and safety

The median resolution day of the fever was 3 (1–10) days after anakinra treatment. The lymphocyte counts, BNP, CRP, and D-dimer values of the patients became normal on the median day of 5 (1–18), 8.5 (5–53), 10 (1–33), and 11.5 (4–20), respectively. The pattern of improvement in EF

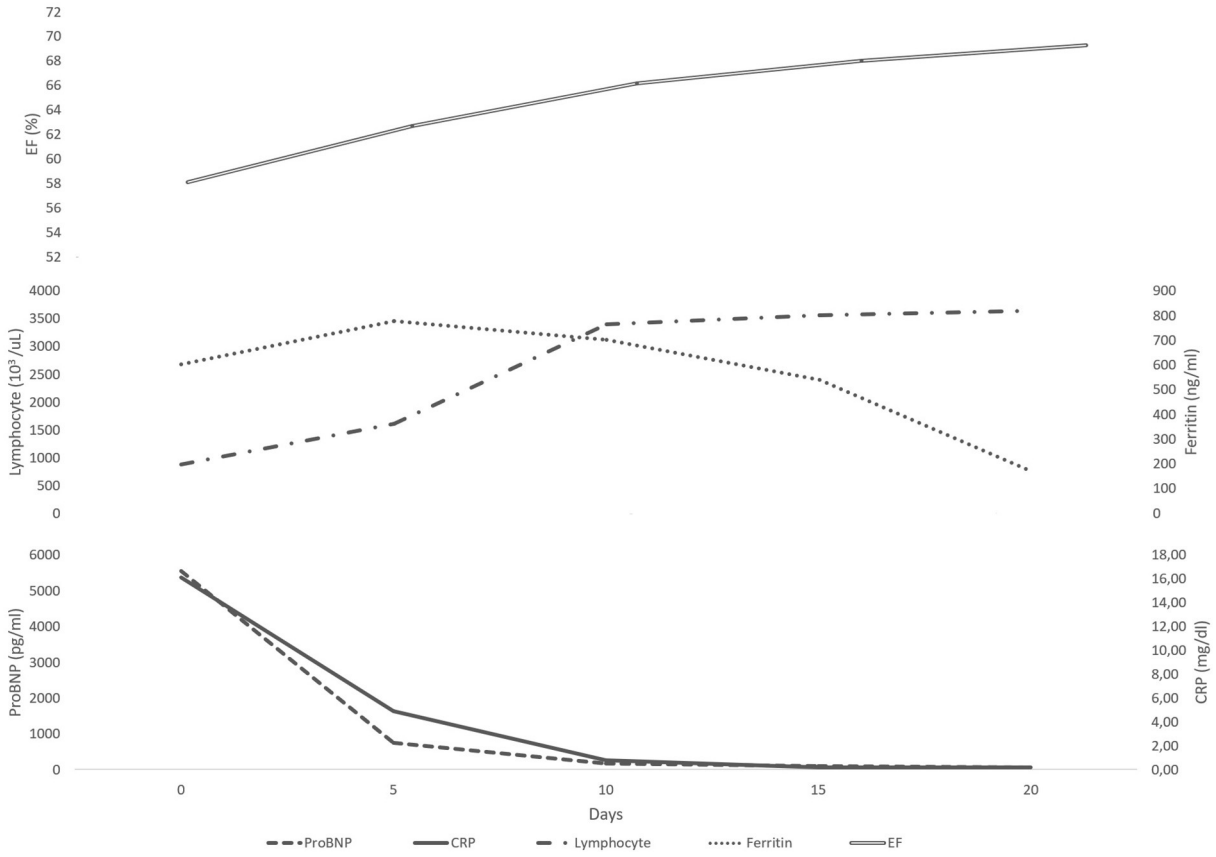

FIGURE 1
The daily variation of median laboratory values under anakinra treatment.

TABLE 3 Side effects observed under anakinra treatment.

Patient number	AE	SAE	Concomitant treatments
Patient 9	AST:123 U/L ALT:174 U/L		Ceftriaxone, noradrenaline, methylprednisolone, LMWH
Patient 26		Acute pancreatitis	Ceftriaxone, noradrenaline, methylprednisolone, LMWH
Patient 38	AST:95 U/L ALT:87 U/L		Ceftazidim, noradrenaline, methylprednisolone, LMWH
Patient 40	AST:142 U/L ALT:102 U/L		Vancomycin, meropenem, noradrenaline, methylprednisolone, LMWH
Patient 53	AST:183 U/L ALT:162 U/L		Cefotaxime, noradrenaline, methylprednisolone, LMWH
Patient 57	AST:137 U/L ALT:221 U/L		Cefotaxime, noradrenaline, methylprednisolone, LMWH
Patient 58	AST:115 U/L ALT:89 U/L		Vancomycin, meropenem, methylprednisolone, LMWH
Patient 59	AST:139 U/L ALT:346 U/L		Ceftriaxone, noradrenaline, methylprednisolone, LMWH
Patient 60	AST:183 U/L ALT:173 U/L		Vancomycin, meropenem, methylprednisolone, LMWH
Patient 63	AST:165 U/L ALT:83 U/L		Cefotaxime, noradrenaline, methylprednisolone, LMWH
Patient 68	AST:102 U/L ALT:92 U/L		Vancomycin, meropenem, noradrenaline, methylprednisolone, LMWH
Patient 70	AST:82 U/L ALT:94 U/L		Cefotaxime, noradrenaline, methylprednisolone, LMWH
Patient 75	AST:771 U/L ALT:859 U/L	Acute cholangitis	Ceftriaxone, noradrenaline, methylprednisolone, LMWH
Patient 78	AST:99 U/L ALT:160 U/L		Vancomycin, meropenem, noradrenaline, methylprednisolone, LMWH

AE, Adverse event; SAE, serious adverse event; LMWH, low-molecular-weight heparin; AST, aspartate aminotransferase (N:5-34 U/L); ALT, alanine aminotransferase (N:0-41 U/L).

and laboratory parameters are shown in Figure 1. None of the patients experienced injection site reactions related to anakinra while elevated transaminase levels were observed in 13 patients (15.8%). Side effects that develop after the use of anakinra and concomitant treatments are shown in Table 3. Overall, after anakinra treatment, 63 patients achieved clinical improvement with anakinra without the need for additional treatments. Nineteen patients required plasmapheresis and 2 patients needed ECMO. Seven patients (8.5 %) died, five of which were due to severe MAS and two due to fulminant myocarditis. Abnormal echocardiographic findings continued in two patients (2.4%) (coronary artery dilatation in one, low ejection fraction in one) at discharge and became normal on the 2nd month.

Discussion

When MIS-C was first defined, IVIG was announced as the main treatment for this new disease because of its similar clinical findings with KD. However, after the clarification of cytokine storm in the pathogenesis of MIS-C, anakinra came to the fore as an alternative treatment in IVIG-resistant cases. Anakinra, a recombinant interleukin (IL)-1 receptor antagonist, is a safely preferred agent in children with MAS. But, the efficacy and safety of anakinra in the treatment of MIS-C is still unclear due to the lack of large controlled clinical trials. We have observed that it was a successful treatment modality in most of the MIS-C patients.

As MIS-C is an emerging phenomenon, new approaches were needed to guide healthcare providers when treating patients. For this purpose, the ACR announced a recommendation set for the management of MIS-C on May 22, 2020 (5). Subsequently, this recommendation set was updated (10). According to the latest version, IVIG and/or corticosteroids are considered first-tier agents and in the presence of a refractory course, high-dose anakinra (>4 mg/kg/day IV or SC) should be initiated (10). The use of anakinra for pediatric inflammatory disease is rapidly expanding with its rapid effect and short half-life. Furthermore, it has a good safety profile even in high doses. In our study, the median dose of anakinra was 2.7 (2–10) mg/kg/day. Depending on the difficulties in the recruitment of anakinra and the immediate treatment needs of the patients, sometimes anakinra vials were shared with more than one patient. Since clinical improvement was observed in the patients, low-dose treatment was continued. All of the patients with poor, rapidly deteriorating general conditions received the recommended high doses of anakinra Although routine administration of anakinra is SC, there are also studies on its IV use. Phadke et al. (11) showed the safety and efficacy of IV anakinra in the treatment of MAS. Cavalli et al. (12) showed that high-dose (10 mg/kg/day) IV anakinra reduced the need for invasive mechanical ventilation or death in patients with hyper-inflammation due to COVID-19. Herein, we presented 82 pediatric MIS-C patients who were treated with a 2.7 mg/kg/day median dose of anakinra while high-dose (>4 mg/kg) anakinra was used in 28 patients (34.1%). All of them were IVIG and corticosteroid resistant and had a severe disease course. Of 82 patients, IV anakinra was used in 12 patients (14.6%). In the presence of thrombocytopenia, subcutaneous edema, or shock, IV administration instead of SC anakinra may be an alternative. In the present study, the patients receiving IV anakinra had left ventricular dysfunction and no anaphylactic reaction was observed. Continuous IV infusion of anakinra provided clinical improvement in 4/5 patients with MAS (13). Furthermore, recently, the efficacy and safety of IV anakinra in patients with non-familial haemophagocytic lymphohistiocytosis (HLH) even in extremely high doses (48 mg/kg/day) has been reported (14). Intravenous anakinra could be an option in selected patients and appears to be well tolerated at high doses.

The studies focusing on anakinra in refractory MIS-C patients are limited. Bhat et al. (15) showed successful outcomes for two refractory MIS-C patients treated with anakinra. Besides anakinra, other biologic drugs such as tocilizumab and infliximab were announced as an alternative option in refractory patients. Compared with other biologic drugs, anakinra's short half-life makes it more suitable. It is well known that anakinra is a safe drug for patients with severe sepsis. Çelikel et al. (16) evaluated the role of biological agents in the treatment of MIS-C. They prescribed anakinra to 23 patients with MIS-C and showed a significant resolution in laboratory parameters (16). Furthermore, successful results with anakinra in patients with the multisystem inflammatory syndrome in adults were published (17).

Injection site reaction is the most common side effect of anakinra treatment (18). However, no injection site reaction was observed in the present study. Cavalli et al. (12) reported elevated transaminase levels (more than three times the upper limit of normal) in 13% of patients with hyper-inflammation due to COVID-19 receiving anakinra. Correspondingly, elevated transaminase levels were observed in 15.8% (n = 13) of our patients. However, it is difficult to clearly state whether this was an adverse effect or was related to the disease course.

According to adult studies, anakinra reduces the need for mechanical ventilation, length of stay in the hospital and intensive care unit, and mortality in patients with COVID-19 (19, 20). But, unfortunately, despite intensive treatment, the mortality rate of MIS-C is reported to range between 1.2 and 1.7% (21, 22). In the present study, seven patients died despite all treatments. The mortality rate in our total MIS-C patients was 1.8%, which is similar to the literature, but the mortality rate in patients using anakinra was 8.5%. Of those who died, one had systemic juvenile idiopathic arthritis and one had Kostmann disease. The other five patients did not have any known comorbidity. Two patients died due to fulminant myocarditis,

and five patients died due to multiorgan failure after MAS. These patients had a severe and rapid disease course from the first admission to the hospital. Pulse corticosteroids, IVIG, and anakinra treatments were applied to all of the patients, five patients underwent plasmapheresis, and ECMO was used in two patients. However, despite all treatments, these patients died. The main limitation of our study is its retrospective design with the absence of a control or placebo group. However, the study supports that anakinra may be an effective treatment option in refractory MIS-C patients.

In conclusion, since the release of interleukin-1 is a key role in the pathogenesis of cytokine storm syndrome, anakinra seems to be the preferred agent in MIS-C patients. According to the results of our study, anakinra could be a successful treatment modality in IVIG and corticosteroid unresponsive, refractory MIS-C cases. Compared to other biologic agents, short half-life of anakinra brings the drug to the stage when quick treatment decisions regarding the patient's treatment is required.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

BS and ŞÇ conceptualized and designed the study, drafted the initial manuscript, and had full access to all the data in the study. All authors conducted the data analyses, drafted the initial manuscript, had full access to all the data in the study, reviewed and revised the manuscript, and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Risk factors for admission to the pediatric critical care unit among children hospitalized with COVID-19 in France

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Background: COVID-19 infection is less severe among children than among adults; however, some patients require hospitalization and even critical care. Using data from the French national medico-administrative database, we estimated the risk factors for critical care unit (CCU) admissions among pediatric COVID-19 hospitalizations, the number and characteristics of the cases during the successive waves from January 2020 to August 2021 and described death cases.

Methods: We included all children (age < 18) hospitalized with COVID-19 between January 1st, 2020, and August 31st, 2021. Follow-up was until September 30th, 2021 (discharge or death). Contiguous hospital stays were gathered in "care sequences." Four epidemic waves were considered (cut off dates: August 11th 2020, January 1st 2021, and July 4th 2021). We excluded asymptomatic COVID-19 cases, post-COVID-19 diseases, and 1-day-long sequences (except death cases). Risk factors for CCU admission were assessed with a univariable and a multivariable logistic regression model in the entire sample and stratified by age, whether younger than 2.

Results: We included 7,485 patients, of whom 1988 (26.6%) were admitted to the CCU. Risk factors for admission to the CCU were being younger than 7 days [OR: 3.71 95% CI (2.56–5.39)], being between 2 and 9 years old

[1.19 (1.00–1.41)], pediatric multisystem inflammatory syndrome (PIMS) [7.17 (5.97–8.6)] and respiratory forms [1.26 (1.12–1.41)], and having at least one underlying condition [2.66 (2.36–3.01)]. Among hospitalized children younger than 2 years old, prematurity was a risk factor for CCU admission [1.89 (1.47–2.43)]. The CCU admission rate gradually decreased over the waves (from 31.0 to 17.8%). There were 32 (0.4%) deaths, of which the median age was 6 years (IQR: 177 days–15.5 years).

Conclusion: Some children need to be more particularly protected from a severe evolution: newborns younger than 7 days old, children aged from 2 to 13 years who are more at risk of PIMS forms and patients with at least one underlying medical condition.

KEYWORDS

COVID-19, SARS-CoV-2, children, critical care, hospitalization

Introduction

Two years after the first cases, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic continues to spread around the world with the regular emergence of new variants of concern. Rapid dissemination of the delta variant (B.1.617.2) in the summer of 2021 and then of the omicron variant (B.1.1.529) in the winter of 2021–2022 were associated with an increase in children hospitalizations, which worries the pediatric community in several affected countries (1–4). Although this surge of pediatric hospitalizations seemed to be mainly attributable to higher contagiousness rather than to higher severity concerning the delta variant (5), the follow-up is ongoing for the omicron variant.

Even if morbidity has been much lower for children than for adults, the pediatric population has not been completely spared (6–14). Children could also be severely affected, requiring hospitalizations and intensive care unit admission, although deaths rarely occur in children (15). Several risk factors associated with these severe forms have already been identified, notably including young age and underlying medical conditions (6, 7, 13–16). Their precise understanding is important in the long-term management of the pandemic, both to identify children who should most benefit from adapted care and surveillance and to design and implement relevant public health policies, insofar as the psychological health of children is affected by these measures (17).

Some studies have already looked at pediatric hospitalizations and risk factors for severity, but none has provided a review of hospitalizations nationwide over a long period and taken into account contiguous hospital stays. Thus, the aim of our national observational study were since the outbreak of COVID-19 (from January 1st, 2020, to September 30th, 2021) to determine the risk factors associated with pediatric critical care unit (CCU) admissions. The secondary aims were to describe the number and characteristics of pediatric hospitalizations in all French hospitals and the clinical characteristics of children who died of COVID-19.

Materials and methods

Study design

We performed a retrospective nested case-control cohort analysis using data from the French national "*Programme de Médicalisation des Systèmes d'Information*" (PMSI) database (18). The PMSI is a comprehensive national database that gathers pseudonymized hospitalization data transmitted monthly by all public and private hospitals in France. Diagnoses are coded using the International Classification of Diseases, 10th Revision (ICD-10).

This study was conducted in accordance with French legislation concerning the reuse of the PMSI database (MR-005 of the Commission nationale de l'informatique et des libertés, CNIL), with an inscription on the Health Data Hub public register (N° F20201117130456). Patients were not involved, as we used pseudonymized discharge data.

We included data from all children (age < 18) admitted to French hospitals with COVID-19 between January 1st, 2020, and August 31st, 2021. Patients were followed up until discharge or death until September 30th, 2021. Hospital stays for COVID-19 were identified according to national guidelines (19; **Supplementary Table 1**). Acute COVID-19 cases were classified into three categories: respiratory form (ICD codes U07.10, U07.11), pediatric inflammatory multisystem syndrome (PIMS) (U10.9 or COVID-19 code associated with M30.3 (mucocutaneous lymph node syndrome [Kawasaki]))

and "neither PIMS nor respiratory form" (U07.14, U07.15). If the case matched with many categories, the prioritization order was PIMS > respiratory form > neither PIMS nor respiratory form. We did not include asymptomatic COVID-19 cases and post-COVID-19 diseases (**Supplementary Table 1**).

All contiguous hospital stays between hospitals for the same patient (<1 day between stays) were gathered and considered a unique "care sequence." The care sequence starting date was the starting date of the first stay, and the ending date was the date of discharge of the last stay. Only the first sequence per patient was

TABLE 1 Characteristics of the children hospitalized with COVID-19.

	All Children (*n* = 7,485)	With CCU (*n* = 1,988)	Without CCU (*n* = 5,497)
Male gender	4,025 (53.8%)	1,115 (56.1%)	2,910 (52.9%)
Age, median (IQR)	1 Year (57 days–10 years)	4 Years (105 days–11 years)	1 Year (53 days–10 years)
Age groups			
0–6 days	130 (1.7%)	68 (3.4%)	62 (1.1%)
7 days to less than 3 months	2,262 (30.2%)	412 (20.7%)	1,850 (33.6%)
3 months to less than 2 years	1,476 (19.7%)	312 (15.7%)	1,164 (21.2%)
2–9 years	1,591 (21.3%)	609 (30.6%)	982 (17.9%)
10–13 years	715 (9.6%)	255 (12.8%)	460 (8.4%)
14–17 years	1,311 (17.5%)	332 (16.7%)	979 (17.8%)
Underlying medical condition			
Respiratory disease	671 (9.0%)	254 (12.8%)	375 (6.8%)
Asthma	468 (6.3%)	186 (9.4%)	282 (5.1%)
Chronic lung disease excluding asthma	203 (2.7%)	86 (4.3%)	117 (2.1%)
Metabolic disease	353 (4.7%)	163 (8.2%)	176 (3.2%)
Diabetes	96 (1.3%)	29 (1.5%)	67 (1.2%)
Metabolic disease excluding diabetes	257 (3.4%)	140 (7.0%)	117 (2.1%)
Sickle-cell disease	211 (2.8%)	90 (4.5%)	121 (2.2%)
Obesity	107 (1.4%)	42 (2.1%)	65 (1.18%)
Cardiovascular disease	275 (3.7%)	143 (7.2%)	132 (2.4%)
Neurologic disease	361 (4.8%)	147 (7.4%)	214 (3.9%)
Immunocompromised condition (including cancers)	363 (4.9%)	129 (6.5%)	234 (4.3%)
Hepatic and gastric disease	48 (0.6%)	14 (0.7%)	34 (0.6%)
Renal disease	45 (0.6%)	15 (0.8%)	30 (0.5%)
Down syndrome	42 (0.6%)	15 (0.8%)	27 (0.5%)
Prematurity among < 2 years (*n* = 3,868)	375 (9.7%)	129 (16.3%)	246 (8.0%)
Number of underlying conditions			
0	5,380 (71.9%)	1,145 (57.6%)	4,235 (77.0%)
1	1,597 (21.3%)	621 (31.2%)	976 (17.8%)
2	367 (4.9%)	162 (8.1%)	205 (3.7%)
3 or more	141 (1.9%)	60 (3.0%)	81 (1.5%)
COVID-19 forms			
PIMS	806 (10.8%)	512 (25.8%)	297 (5.4%)
Respiratory form	3,294 (44.0%)	838 (42.2%)	2,456 (44.7%)
No PIMS no respiratory form	3,385 (45.2%)	638 (32.1%)	2,747 (50.0%)
Level of care required			
Admission in critical care unit	1,988 (26.6%)		
Ventilation	461 (6.2%)	387 (19.5%)	74 (1.3%)
Invasive ventilation	149 (2.0%)	133 (6.7%)	16 (0.3%)
Non-invasive ventilation	385 (5.1%)	320 (16.1%)	65 (1.2%)
ECMO	7 (0.1%)	7 (0.4%)	0 (0.0%)
Need of vasoactive drugs	241 (3.2%)	204 (10.3%)	37 (0.7%)
Hospital length of stay, median (IQR), days	3 (1–5)	6 (3–10)	2 (1–4)

IQR, interquartile range; CCU, critical care unit; ECMO, extracorporeal membrane oxygenation; PIMS, pediatric multisystem inflammatory syndrome.

considered. In the context of COVID-19, France has adopted a massive screening strategy and these involved often scheduled stays with systematic screening outside the scope of our study. Consequently, we excluded care sequences lasting less than a day, except in cases of death.

Outcomes

The primary outcome was the requirement for hospitalization in CCUs. The CCUs included intensive care units, intermediate care units (so-called "*soins intensifs*"), and step-down units (so-called "*unité de surveillance continue*"). The secondary outcome was in-hospital death.

Covariates

The variables extracted for each patient were age, sex, dates of hospital admission and discharge, length of stay, chronic conditions, and admission to a CCU. The use of invasive or non-invasive ventilation, extracorporeal membrane oxygenation (ECMO), and vasoactive drugs were defined based on the act coded using the Common French Classification of Medical Acts (**Supplementary Table 2**).

The ICD-10 codes used to specify underlying chronic conditions were defined by a team of physicians experienced in medical information and a pediatrician and are listed in **Supplementary Table 3**. These chronic conditions were retrieved from the current care sequence and from the hospitalizations within the two previous years. The following 12 chronic conditions were considered: asthma, chronic lung disease excluding asthma, diabetes, metabolic disease excluding diabetes, sickle cell disease, obesity, cardiovascular disease, neurologic diseases, immunocompromised conditions including cancer, Down syndrome, hepatic and gastric disease, and renal disease. In addition, for children younger than 2 years old, prematurity was considered when one hospitalization within the previous 2 years contained one of the following ICD10 codes (P07.2, P07.3) or when the birth term was lower than 37 amenorrhea weeks.

Statistical analysis

For continuous variables, data were described by their median and interquartile ranges [IQRs]. Categorical variables were described as numbers of patients and percentages. Age was also divided into age groups: 0–7 days, 8–89 days, 90 days-1 year, 2–9 years, 10–13 years, and 14–17 years. For global chronological description, the time unit used was the month of the sequence's starting date. Four periods, corresponding to 4 different epidemic waves, were considered from January 1st to August 11th 2020, from August 12th 2020 to January 1st 2021, from January 2nd to July 4th 2021 and from July 5th to August 31st 2021.

Qualitative variables were compared between groups using Chi-square tests, and quantitative variables were compared using ANOVA. Risk factors for CCU admission were assessed with a univariable and a multivariable logistic regression model in the entire sample and stratified by age, whether younger than 2. Multivariable models included all the variables with at least 10 patients per modality.

The analyses were performed on the secure platform of the ATIH. Data extraction and preparation were carried out on January 11th 2022 with SAS Enterprise Guide version 8.3. *P*-values lower than 0.05 were considered statistically significant.

Results

Study population

We identified 11,414 patients younger than 18 years who were hospitalized with SARS-CoV-2 infection in France between March 2020 and August 2021. We excluded 1,848 (16.2%) patients who were discharged alive after a length of stay less than 1 day, 1,972 (17.3%) patients with asymptomatic infections and 109 (1.0%) hospitalizations related to post-COVID symptoms. The characteristics of the excluded patients are given in **Supplementary Table 4**. They were more frequently younger than 6 days and older than 10 years old than the included patients. In addition, they had less comorbidities, except for diabetes that was twice more frequent. Finally, 7,485 children were included, all of whom had been admitted to a hospital with a symptomatic SARS-CoV-2 infection. The median duration of hospitalization was 3 days (IQR 1–5); 1,988 (26.6%) were admitted to CCUs, and 32 (0.4%) died (**Figure 1**).

Demographic and clinical characteristics

The median age of symptomatic hospitalized children was 1.0 year (IQR: 57 days-10 years); 32.0% were under 3 months old (n = 2,392) (**Table 1**). Most children were male (53.8%) (**Figure 2** and **Supplementary Figure 1**). No underlying medical condition was identified in 71.9% of hospitalized children, even though this proportion varied in the different age groups to be minimal for the 2–9-year-old group (61.2%). For all these children, the most common underlying condition was chronic lung disease, including asthma (9.0%), followed by immunocompromised conditions (4.9%), neurologic diseases (4.8%) and metabolic disease (4.7%). Other underlying conditions appear in **Table 1**. Prematurity was reported for 375 children younger than 2 years old, i.e., 9.7% of this age group.

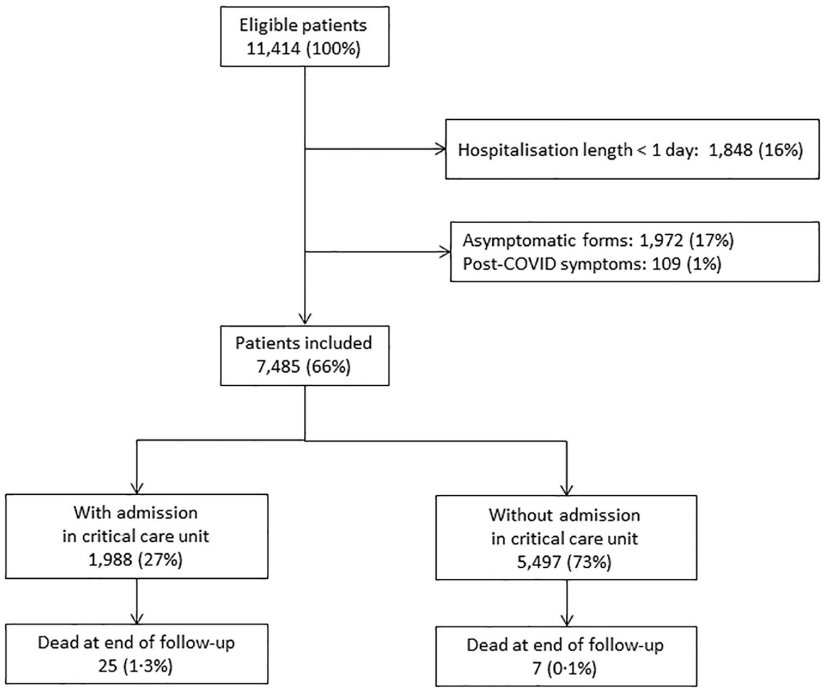

FIGURE 1
Study patient selection flow chart.

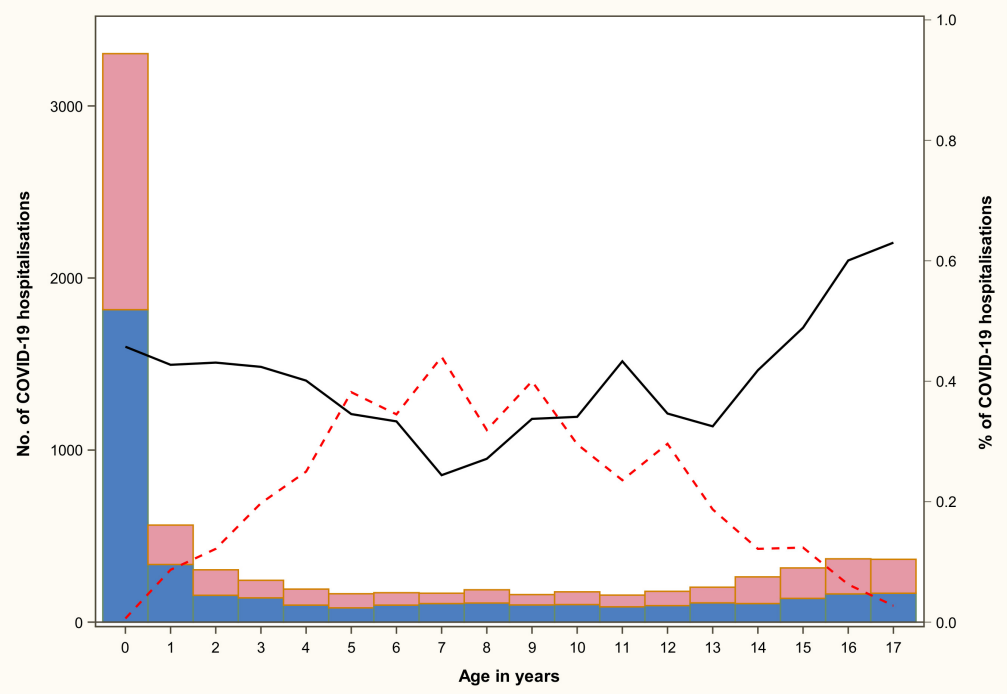

FIGURE 2
Number of COVID-19 hospitalizations and of the PIMS and respiratory forms according to the age and gender of the patient. The bars give the number of COVID-19 hospitalizations for the males (blue) and the females (pink) separately. The lines give the proportion of the PIMS (red dotted line) and respiratory (black plain line) forms among the hospitalizations.

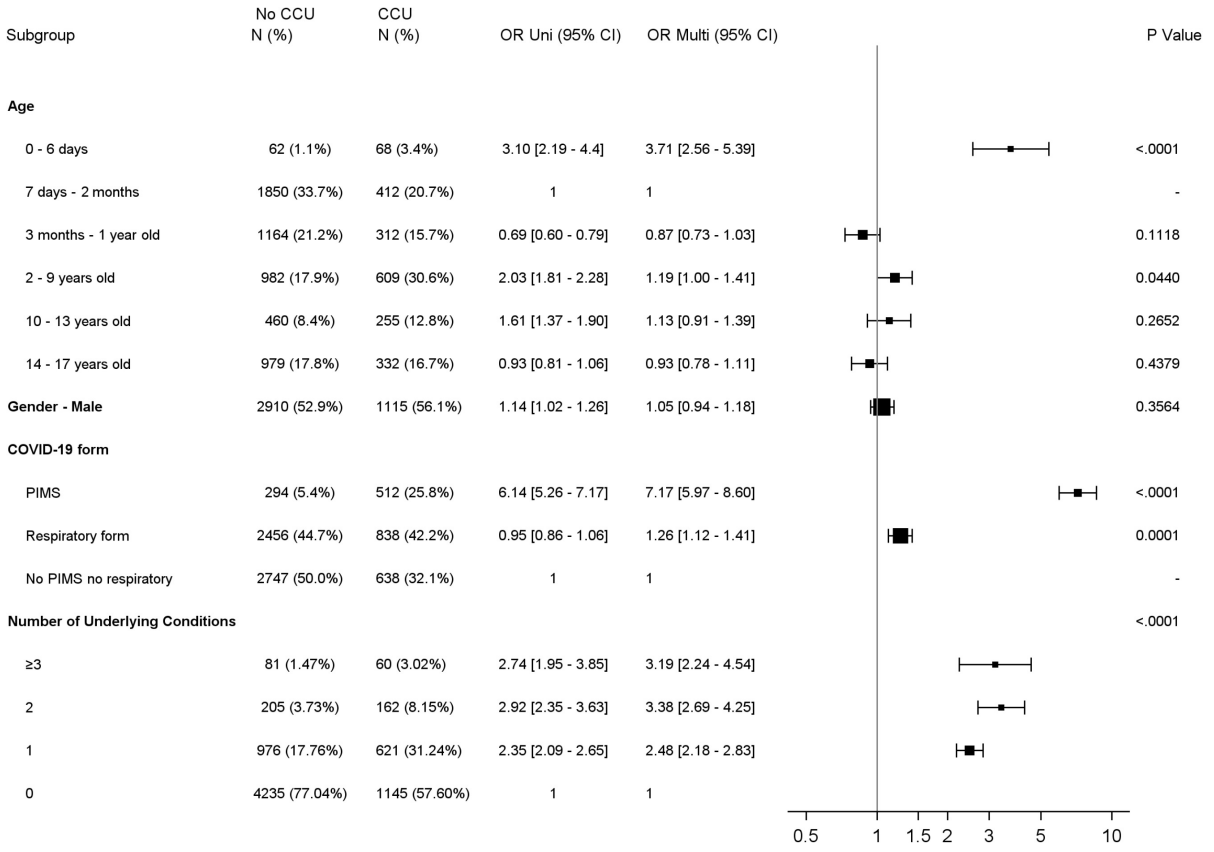

FIGURE 3
Forest plot showing the odds ratio using Univariable (Uni) and Multivariable (Multi) Logistic regression analysis for CCU admission for all children. CCU, critical care unit; PIMS, pediatric multisystem inflammatory syndrome; OR, Odds ratio; 95% CI, 95% confidence interval.

COVID-19 forms

Respiratory forms represented 44.0% of hospitalizations (n = 3,294). The PIMS rate was 10.8% (n = 806) for all admitted children, with a maximum rate of 44.0% for the 7-year-old children (Figure 2). In fact, the 2–9-year-old group accounted for 56.2% (n = 453) of all PIMS. The proportion of PIMS and respiratory forms of COVID-19 hospitalized by age is shown in Figure 2. The demographic and clinical characteristics of the different forms of COVID-19 are presented in Supplementary Table 5.

Risk factors for critical care unit admission

Over the entire analysis period, 461 (6.2%) children required ventilatory support (Table 1). Seven children were treated by ECMO. The use of vasoactive drugs was reported for 241 patients (3.2%), among whom 171 (71.0%) had PIMS. Risk factors for admission to CCUs evidenced by multivariate analyses (Figure 3 and Supplementary Table 6) included age younger than 7 days old [OR: 3.71 95% CI (2.56–5.39)] and age between 2 and 9 years old [1.19 (1.00–1.41)]; and PIMS [7.17 (5.97–8.6)] and respiratory forms [1.26 (1.12–1.41)] compared to "neither PIMS nor respiratory form." The presence of at least one underlying condition increased the risk of CCU admission [2.66 (2.36–3.01)]. Sex was not associated with CCU admission [1.05 (0.94–1.18)].

As prematurity was assessed only for children younger than 2 years old, the analysis of risk factors for CCU admission was stratified according to the age of children (<> 2 years old). Among hospitalized children younger than 2 years old, most underlying conditions identified were significantly associated with CCU admissions except for Down syndrome, chronic lung disease excluding asthma, and neurologic and metabolic disease (Figure 4 and Supplementary Table 7). Prematurity, the most common underlying condition in this age group (16.3% of those admitted to the CCU), was identified as a risk factor for CCU admission [1.89 (1.47–2.43)].

Among those older than 2 years old, metabolic disease [2.97 (2.15–4.08)], sickle cell disease [2.70 (1.97–3.70)], asthma [2.10 (1.64–2.69)], cardiovascular disease [2.10 (1.45–3.03)], obesity [2.06 (1.36–3.13)] and neurologic disease [1.84 (1.40–2.43)]

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TABLE 2 Characteristics of the deceased children hospitalized with COVID-19.

	Deceased (n = 32)
Male gender	16 (50.0%)
Age, median (IQR)	6 Years (177 days–15.5 years)
Age groups	
0–6 days	4 (12.5%)
7 days to less than 3 months	1 (3.1%)
3 months to less than 2 years	8 (25.0%)
2–9 years	5 (15.6%)
10–13 years	2 (6.3%)
14–17 years	12 (37.5%)
Underlying medical condition	
Respiratory disease	10 (31.3%)
Asthma	4 (12.5%)
Chronic lung disease excluding asthma	6 (18.8%)
Metabolic disease	7 (21.9%)
Diabetes	0
Metabolic disease excluding diabetes	7 (21.9%)
Sickle-cell disease	0
Obesity	1 (3.1%)
Cardiovascular disease	5 (15.6%)
Neurologic disease	19 (59.4%)
Immunocompromised condition (including cancers)	6 (18.8%)
Hepatic and gastric disease	0
Renal disease	0
Down syndrome	0
Prematurity among < 2 years (n = 3868)	5 (38.5%)
Number of underlying conditions	
0	4 (12.5%)
1	13 (40.6%)
2	8 (25.0%)
3 Or more	7 (21.9%)
COVID-19 forms	
PIMS	1 (3.1%)
Respiratory form	25 (78.1%)
No PIMS no respiratory form	6 (18.8%)
Level of care required	
Admission in critical care unit	25 (78.1%)
Ventilation	23 (71.9%)
Invasive ventilation	20 (62.5%)
Non-invasive ventilation	9 (28.1%)
ECMO	4 (12.5%)
Need of vasoactive drugs	12 (37.5%)
Hospital length of stay, median (IQR), days	8 (2–20.5)

IQR, interquartile range; CCU, critical care unit; ECMO, extracorporeal membrane oxygenation; PIMS, pediatric multisystem inflammatory syndrome.

significantly increased the risk of CCU admission (Figure 5 and Supplementary Table 7). In this group, Down syndrome and chronic lung disease excluding asthma were not found to be at risk either.

Pediatric deaths

Among the children in the study, 32 (0.4%) died during their hospital stay (Table 2). The median age of the deceased patients was 6.0 years (IQR: 177 days–15.5 years). Four had no underlying condition identified, and among the 28 others (87.5%), neurologic disorders were the most common (n = 19, 59.4%), followed by chronic lung disease (n = 10 including 4 with asthma, 31.3%), metabolic disease (n = 7, 21.9%) and immune deficiency (n = 6, 18.8%). Five of those who died had a cardiovascular disease (15.6%), and one was obese (3.1%). None had Down syndrome, sickle cell disease, or a chronic renal or digestive disease. Among the children younger than 2 who died (n = 13), 5 were premature. Regarding the distribution over time, 14 deaths occurred during the first wave (until August 2020), 5 during the second wave, 7 during the third wave and 6 during the fourth wave.

Hospitalizations over time (waves)

Symptomatic COVID-19 admissions among children in French hospitals peaked in March 2020 (first wave), October 2020 (second wave), April 2021 (third wave) and August 2021 (fourth wave), with 478, 648, 713, and 923 monthly admissions, respectively (Figure 6). The percentage of hospitalizations with admission to CCUs among all hospitalizations gradually decreased over the different waves: Starting with 31.0% admitted to CCU, then 27.6, 27.5, and 17.8%, respectively. The mean age decreased from 6.0 ± 0.2 years old during the first wave to 3.8 ± 0.2 years old during the fourth wave ($p < 0.001$).

Discussion

The national PMSI database enabled the exhaustive analysis of 7,485 pediatric hospitalizations for symptomatic SARS-CoV-2 infection from January 2020 to September 2021 in France. This study, gathering the largest number of symptomatic children hospitalized over the longest period ever assessed, strengthens knowledge of pediatric COVID-19 and highlights risk factors for severe disease. More than one-fourth of these children required admission to the CCU, and risk factors identified by multivariate analyses were (i) being younger than 7 days old or from 2 to 9 years old and (ii) PIMS or "respiratory form." Most underlying conditions were significantly associated with CCU

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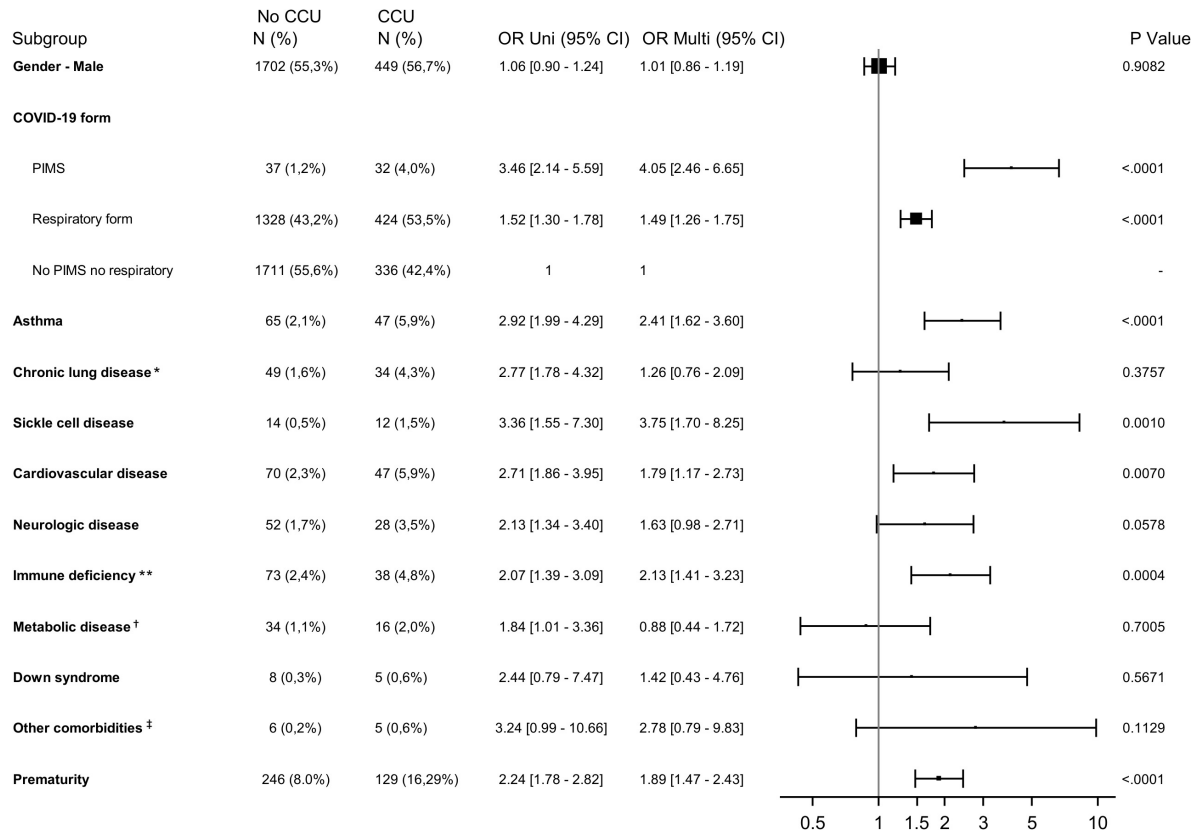

FIGURE 4
Forest plot showing the odds ratio using univariable (Uni) and multivariable (Multi) logistic regression analysis for CCU admission for children younger than 2 years old. CCU, critical care unit; PIMS, pediatric multisystem inflammatory syndrome; OR, odds ratio; 95% CI, 95% confidence interval. *Excluding asthma; **including cancers; †excluding diabetes; ‡renal disease, hepatic and gastric disease, and diabetes.

admissions. Unlike adults, Down syndrome and diabetes were not significantly associated (20, 21).

The rate of admissions to the CCU (26.6%) was in the upper range of those reported in similar pediatric studies between 4.1 and 30.1% (5, 7, 13, 22). This high rate may be partly explained by the methodology of this study and the choice to exclude asymptomatic hospitalized COVID-19 children, who were considered incidental findings. Another explanatory factor relates to the organization of care in France at the beginning of the pandemic, when all children infected by SARS-CoV-2 and requiring hospitalizations were grouped together in dedicated wards within the CCU. This is supported by the highest rate of hospitalizations in the CCU during the first wave in France (31%), when the traditional care organization was redeployed. Overall, this decrease in the CCU rate over time is another reassuring element in the analysis of the severity of the various variants of concern.

The analysis of CCU admission revealed an increased risk for patients younger than 7 days old. The fragility of this age group is presumably partly linked to particular neonatal contexts (prematurity, fetal distress, etc.) (23). Indeed, 37% of the hospitalized infants younger than 7 days old were premature. The involvement of SARS-CoV-2 infection in the vulnerability of these new-borns is not certain but should nevertheless attract our attention when it also appears that neonates without any history or underlying condition can present severe forms such as pneumonia (23, 24). This fragility was not confirmed in our analysis for infants older than 7 days old, while children younger than 2 years old represented more than half of the hospitalizations in this study. The follow-up of more than a year and a half in our study invalidated the first worrisome data considering infants as a vulnerable group (9). Regarding the 2–9-year-old and 10–13-year-old groups, we found higher odds of admission to the CCU in the univariate analysis, similar to another study (25). However, this was not confirmed by multivariate analyses, and it appeared that these age groups were more often affected by PIMS and therefore more at risk of requiring CCU without having a real over-risk (16, 22, 26).

The analysis of underlying respiratory conditions did not reveal any excess risk of CCU associated with chronic respiratory diseases other than asthma, and the involvement of these comorbidities in severe forms of infection is not

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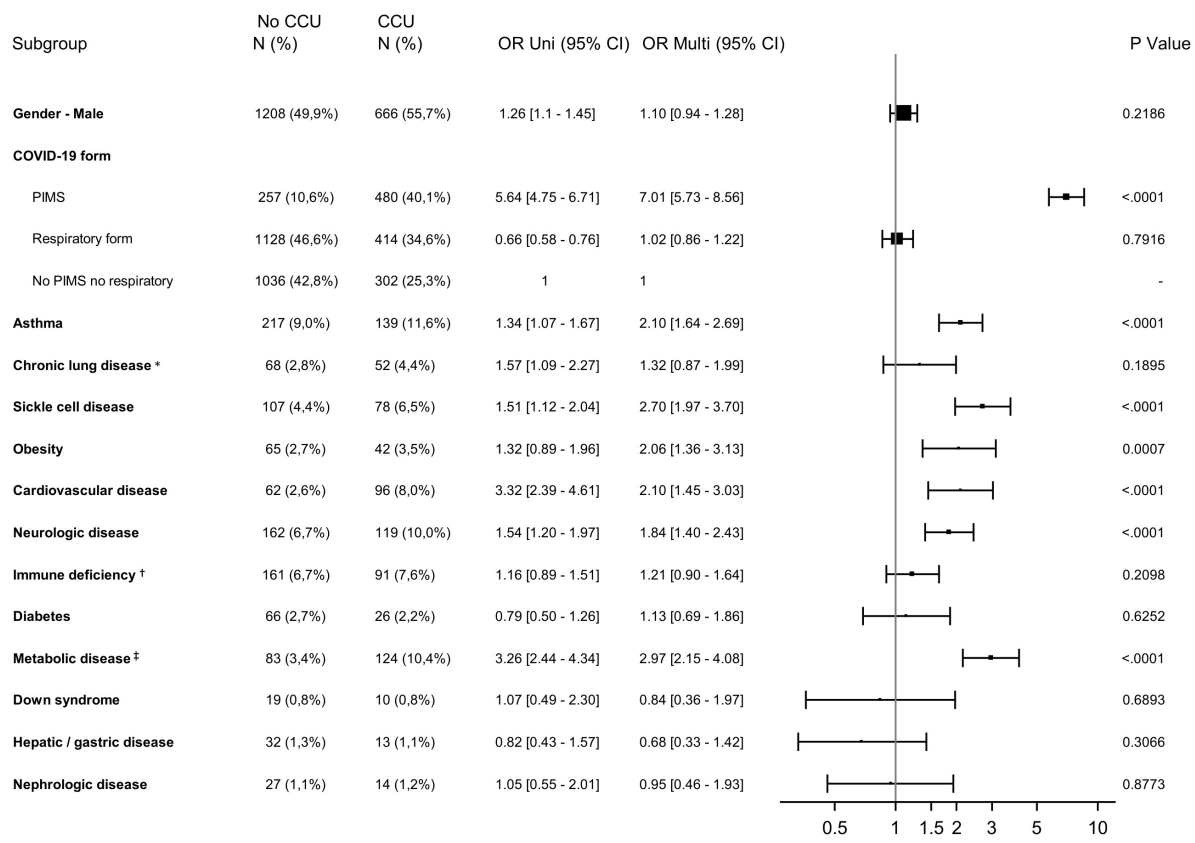

FIGURE 5

Forest plot showing the odds ratio using univariable (Uni) and multivariable (Multi) logistic regression analysis for CCU admission for children older than 2 years old. CCU, critical care unit; PIMS, pediatric multisystem inflammatory syndrome; OR, odds ratio; 95% CI, 95% confidence interval. *Excluding asthma; †including cancers; ‡excluding diabetes.

yet clearly documented for all of them (7). Among patients with chronic respiratory diseases, those with cystic fibrosis were observed in several studies, and the increased risk associated with this pathology mainly concerned the most severe or the posttransplant patients (27). Moreover, these stages of disease concern more adults than children. Asthma was identified here as a risk factor for CCU admission for both age groups (<> 2 years old). Recent studies have also shown the involvement of asthma in the risk of hospital admission but not of CCU admission (7, 28). This increased risk of hospitalization, primarily for children with poorly controlled asthma (29), was not related to asthma severity, and SARS-CoV-2 infection did not appear to be a trigger exacerbation of asthma (28). Our study also gathered 211 children with sickle cell disease. France is the country with the highest prevalence of sickle cell disease in Europe, and we were able to highlight that this disease was associated with a significantly increased risk of CCU admission for children (30). Clinical courses were favorable for all patients. More generally, we have demonstrated a significant excess risk of CCU for children with at least one underlying medical condition. The highlighted underlying conditions can vary among the different previous studies, depending on the proportions of each pathology in the different countries and the differences in underlying condition definitions in the studies. It should be remembered that a child followed for a chronic pathology is more at risk of presenting with severe COVID-19 (7, 13, 14, 16).

In pediatrics, the youngest children, including new-borns and those with comorbidities, are the most vulnerable to respiratory infections (31, 32), and SARS-CoV-2 infection is no exception to the rule. Those children are more at risk of presenting severe complications and of admission to the CCU. The unusual aspect of this pandemic in pediatrics is that healthy middle-aged children can develop serious complications of SARS-CoV-2 infection, such as PIMS, and be hospitalized to CCU. In addition, long COVID-19, which was not described in this study, also seems to affect healthy children older than 6 years old (33). Highlighting these pediatric specificities should encourage families and health professionals to vaccinate all children and pregnant women, above all those with underlying conditions. The safety and efficiency of the BNT162b2 COVID-19 vaccine is high, and very rare adverse

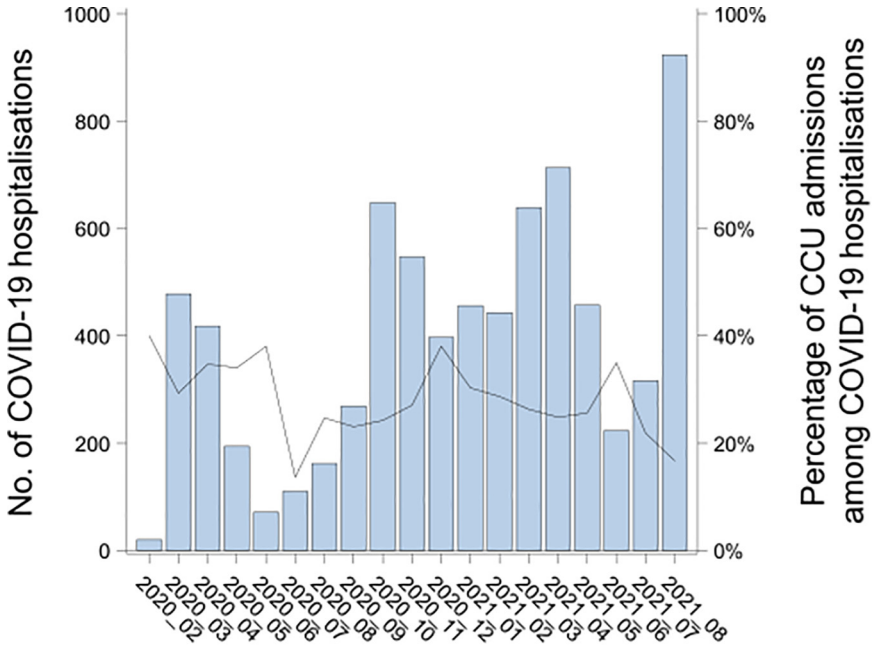

FIGURE 6
Number of pediatric COVID-19 hospitalizations by month and percentage of COVID-19 hospitalizations in critical care units in France from February 2020 to August 2021. The bars give the number of COVID-19 hospitalizations per month. The black plain line gives the percentage of CCU admissions among the COVID-19 hospitalizations.

effects were indeed observed (34). In France, convincing parents to provide COVID-19 vaccination for children aged 5–11 years old is a struggle.

A limitation of the PMSI national database is the possible variation in the quality of coding. We may be confident in the quality of the collected data because national COVID-19 coding guidelines have been published shortly after the beginning of the pandemic. Moreover, strict national rules with regular checks carried out by the payer may limit the risk of coding errors. The PMSI database does have many advantages, such as being exhaustive and national, including all the data from public and private hospitals. In addition, this study allowed us to gather continuous stays within different hospitals so that the risk of overestimating the number of hospitalizations is limited. We included all patients presenting any symptoms of COVID-19 regardless of the severity during their stay if an ICD-10 code was present in their records. If the patients became symptomatic after admission, then an ICD-10 code of symptomatic COVID-19 was present in the file and the patient was considered.

Conclusion

In conclusion, the SARS-CoV-2 pandemic among children is not comparable to the adults' in terms of hospitalization and mortality rates. Nevertheless, childhood morbidity is not negligible, as evidenced by the number of hospitalizations for symptomatic forms in France, the high rate of CCU admission and the number of deaths. We are able to confirm that some children are particularly at risk of evolving toward a severe infection: new-borns younger than 7 days old, children aged from 2 to 13 years who are more at risk of PIMS, and patients with at least one underlying medical condition. Repercussions on the child's overall health are also a constant concern of pediatricians and should of course guide the long-term management of the pandemic.

Data availability statement

Raw data are available upon request to the Agence technique de l'information sur l'hospitalisation (ATIH): support@atih.sante.fr. Aggregated data can be provided upon request to the authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

BP, AR, FB-F, PT, and ST involved in the methodology, formal analysis, investigation, data curation, writing the original draft, reviewing and editing the manuscript, designing of tables and graphs, and verified the underlying data. ABo, ABr, HC, VG, ML, XL, JM, EO, and FS involved in data provision and reviewing and editing the manuscript. All authors had full access to all data in the study and accept responsibility for the decision to submit for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.975826/full#supplementary-material

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Immune and pathophysiologic profiling of antenatal coronavirus disease 2019 in the GIFT cohort: A Singaporean case-control study

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COVID-19 can be severe in pregnant women, and have adverse consequences for the subsequent infant. We profiled the post-infectious immune responses in maternal and child blood as well as breast milk in terms of antibody and cytokine expression and performed histopathological studies on placentae obtained from mothers convalescent from antenatal COVID-19. Seventeen mother-child dyads (8 cases of antenatal COVID-19 and 9 healthy unrelated controls; 34 individuals in total) were recruited to the Gestational Immunity For Transfer (GIFT) study. Maternal and infant blood, and breast milk samples were collected over the first year of life. All samples were analyzed for IgG and IgA against whole SARS-CoV-2 spike protein, the spike receptor-binding domain (RBD), and previously reported immunodominant epitopes, as well as cytokine levels. The placentae were examined microscopically. The study is registered at clinicaltrials.gov under the identifier NCT04802278. We found high levels of virus-specific IgG in convalescent mothers and similarly elevated titers in newborn children. Thus, antenatal SARS-CoV-2 infection led to high plasma titers of virus-specific antibodies in infants postnatally. However, this waned within 3–6 months of life. Virus neutralization by plasma was not uniformly achieved, and the presence of antibodies targeting known immunodominant epitopes did notassure neutralization. Virus-specific IgA

levels were variable among convalescent individuals' sera and breast milk. Antibody transfer ratios and the decay of transplacentally transferred virus-specific antibodies in neonatal circulation resembled that for other pathogens. Convalescent mothers showed signs of chronic inflammation marked by persistently elevated IL17RA levels in their blood. Four placentae presented signs of acute inflammation, particularly in the subchorionic region, marked by neutrophil infiltration even though > 50 days had elapsed between virus clearance and delivery. Administration of a single dose of BNT162b2 mRNA vaccine to mothers convalescent from antenatal COVID-19 increased virus-specific IgG and IgA titers in breast milk, highlighting the importance of receiving the vaccine even after natural infection with the added benefit of enhanced passive immunity.

KEYWORDS

SARS-CoV-2, antenatal COVID-19, transplacental antibody transfer, breast milk antibodies, placental inflammation, GIFT

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has infected over 497 million worldwide (WHO website, as of 13th April 2022) and led to a pandemic. In the midst of this unprecedented crisis, studies on vulnerable groups such as pregnant women and newborns are limited compared to the general adult population. Although the manifestation of COVID-19 is less severe in the pediatric population compared to adults, infants are particularly susceptible to developing severe illness (1). Additionally, concerns over mother-to-child transmission of the virus have led to variable recommendations on postnatal care (2). Human breast milk (BM) is the main source of nutrients and bioactive factors that protects infants against general infections (3). Maternal antibodies which are present in abundance in BM are able to confer protection against specific infections through their antigen specificity. These maternal antibodies comprise of approximately 90% immunoglobulin A (IgA), 8% IgM and 2% IgG (4). SARS-CoV-2 antibodies from convalescent COVID-19 plasma have been extensively studied as a therapeutic option against COVID-19 infection (5, 6). Several studies have also reported the presence of SARS-CoV-2 antibodies in the BM of convalescent COVID-19 mothers (7–9). However, the durability of those antibodies in the BM and the mechanism of protection remains incompletely known. Hence, we sought to evaluate the durability and neutralization capacity of SARS-CoV-2 specific IgG and IgA in the BM of convalescent COVID-19 mothers.

Here, we report that natural infection of pregnant women by SARS-CoV-2 induced antibody production and secretion into maternal blood and milk. However, plasma antibody titers were not closely reflected by that in milk. Placentae from COVID-19 positive mothers exhibited signs of acute inflammation with neutrophilic involvement, despite virus clearance in the nasopharynx. Virus neutralization by plasma was not uniformly achieved, despite high levels of antibodies targeting the spike protein and its receptor-binding domain; the presence of antibodies targeting known immunodominant epitopes did not assure neutralization. Antibody transfer ratios and decay kinetics of virus-specific antibodies in neonatal circulation largely resembled that described for other pathogens. Targeted analysis of blood cytokines revealed significant elevations in IL17RA levels in convalescent mothers' blood at 16 weeks (range 13–48) from the initial COVID-19 infection, indicating chronic inflammation. Importantly, we also found that a single regular dose of the Pfizer/BioNTech BNT162b2 mRNA vaccine given postnatally boosted milk-borne virus-specific IgG and IgA in mothers convalescent from antenatal COVID-19.

Materials and methods

Ethics statement

This study was approved by the National Healthcare Group Institutional Review Board (Gestational Immunity For Transfer GIFT: DSRB Reference Number: 2020/00483). Written informed consent was obtained from all subjects (and where applicable, parents), and the study was conducted in accordance with the Helsinki Declaration. The study protocol was registered at clinicaltrials.gov (NCT04802278).

Collection of human breast milk

Breast milk from study participants were collected at 1 and 3 months postpartum. Milk samples were aliquoted and frozen at –20 degrees Celsius until use. Recruited mothers who were convalescent from antenatal COVID-19 (n = 8 provided blood samples; n = 6 provided BM samples) were confirmed to be positive for COVID-19 infection with real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay on nasopharyngeal swabs during pregnancy. Recovery was defined by the resolution of clinical symptoms and two negative SARS-CoV-2 RT-PCR swabs 24 h apart. Infants born to antenatal COVID-19 convalescent mothers at gestational age between 35 to 40 weeks gestation were included. Mother-child control dyads (n = 9) were also recruited. These control mothers had no clinical symptoms of COVID-19 infection and were confirmed to have a negative SARS-CoV-2 IgG at the time of recruitment.

Blood processing for plasma

Venous blood samples from the infant were collected at 0–3 days, 1 and 3 months of life, and from the mothers at 1 month postpartum, in BD Vacutainer cell preparation tubes (BD, #362753). The plasma fraction was harvested after centrifugation at 1,500 rpm, 5 min at 4oC and stored at –80°C. Sample viral inactivation was performed by treatment with 1% Triton X-100 (Thermo Fisher Scientific, #28314) and 0.3% (w/w) tri-(n-butyl) phosphate (TNBP) at room temperature for 2 h.

Synthesis of the severe acute respiratory syndrome coronavirus 2 receptor binding domain and spike protein

SARS-CoV-2 spike and RBD were generously provided by the Antibody Engineering Programme, Life Sciences Institute, NUS as described previously (10).

Spike and receptor-binding domain-protein based enzyme-linked immunosorbent assays

IgA and IgG against SARS-CoV-2 antigens including the whole spike and RBD protein were titrated using quantitative enzyme-linked immunosorbent assay (ELISA). 96-well flat-bottom maxi-binding immunoplate (SPL Life Sciences, #32296) were coated with 100 ng of SARS-CoV-2 whole spike protein or 200 ng of RBD protein at 4°C overnight. After three washes in Phosphate Buffer Saline (PBS), 350 μL of blocking buffer [4% skim milk in PBS with 0.05% Tween 20 (PBST)] was added to each well. After incubation for 1.5 h, the plate was washed three times with PBST. 100 μL of 10-times diluted human milk samples, or 100-times diluted human plasma samples were added to each well for 1-h incubation. Plate was then washed three times with PBST followed by 1-h incubation in the dark with 100 μL of 5,000-times diluted goat anti-human IgG-HRP (Invitrogen, #31413), or 5,000-times diluted F(ab')2 anti-human IgA-HRP (Invitrogen, #A24458). Plate was washed three times in PBST and incubated for 3 min with 1-Step Ultra TMB-ELISA (Thermo Scientific, #34029), 100 μL per well. Reaction was stopped with 100 μL of 1 M H_2SO_4 and optical density at 450 nm (OD_{450}) was measured using a microplate reader (Tecan Sunrise). OD_{450} was calculated by subtracting the background signal from sample binding to the blocking buffer. Experiments were performed at least three times.

Pseudovirus neutralization test

The pseudovirus neutralization test (PVNT) assay was conducted as described previously (11). Briefly, the ACE2 stably expressed CHO cells were cultured at 5 × 10^4/ml cells in complete medium for 24 h. 80-times diluted plasma were incubated with 50,000 lentiviral particles (representative of the SARS-CoV-2 Wuhan-Hu-1 strain) in a total volume of 50 μL at 37 °C for 1 h. This mixture was added to the CHO-ACE2 monolayer cells and left incubated for 1 h to allow pseudotyped viral infection. Subsequently, complete medium was added at 150 μL/well for further incubation of 48 h. After two washes with PBS, 100 μL of ONE-gloTM EX luciferase assay reagent (Promega, #E8130) was added to each well and the luminescence values were recorded. The percentage neutralization was calculated as follows:

$$\text{Neutralization\%} = \frac{Readout\ (unknown) - Readout\ (infected\ control)}{Readout\ (uninfected\ contorl) - Readout\ (infected\ control)} \times 100\%$$

Breastmilk/plasma inactivation and peptide based enzyme-linked immunosorbent assay

Both plasma and BM samples were inactivated with Triton^{TM} X-100 (Thermo Fisher Scientific, #28314) to a final concentration of 1% for 2 h at room temperature (RT). A focused epitope screen was performed according to a previously described peptide-based ELISA (12). S14P5, S20P2, S21P2 and N4P5 are 18-mer peptides derived from SARS-CoV-2 spike protein with immunodominant activities (12, 13). For

BM ELISA, Streptavidin coated plates (Life Technologies Pierce, #15126) were coated overnight at 4 degrees Celsius with 10 μg/ml peptides diluted in 0.01% polyvinyl alcohol (PVA). Plates were blocked with 0.01% PVA at RT prior to the addition of BM at 1:10 dilution. Goat HRP-conjugated anti-human IgA (Abcam, #ab97215) diluted at 1: 2,000 in the blocking buffer was used for the detection of peptide specific antibodies. TMB substrate (eBioscience, #00-4201-56) was added to the plate for development and the reaction was quenched with 2M sulfuric acid. Absorbance measurements were read at two wavelengths (450 and 570 nm) using the Infinite M200 plate reader (Tecan, firmware V_2.02_11/06). Plates were incubated at RT for 1 h on a rotating shaker for all steps unless otherwise stated and washed thrice with 0.1% PBST in between steps.

For plasma ELISA, Maxisorp plates (Thermo Fisher Scientific, #442404) were coated overnight at 4 degrees Celsius with 1 μg/ml of Neutravidin (Thermo Fisher Scientific, #31050) diluted in PBS. Plates were blocked with 0.01% PVA followed by the addition of 1:100 inactivated plasma samples. The subsequent steps followed the same sequence as mentioned above.

Multiplex microbead-based immunoassay

Quantification of cytokine levels in the plasma samples of convalescent and healthy mothers was performed by multiplex microbead-based immunoassays. Plasma samples were treated with 1% Triton™ X-100 solvent detergent for virus inactivation (14). Concentrations of immune mediators were determined using the Luminex™ assay (HCYTOMAG-60K-41plex) (Millipore Merck). Plasma from participants (n = 7 from each group) and standards were incubated with fluorescent-coded magnetic beads pre-coated with capture antibodies in a 96-well plate. Biotinylated detection antibodies were incubated with the cytokine-bound beads for an hour. Streptavidin-PE was then added for another 30 min before the acquisition of data using xPONENET® 4.0 (Luminex Corporation, USA) software. Data was analyzed using the Bio-Plex Manager™6.1.1. Standard curves were generated with a 5-PL (5-parameter logistic) algorithm, reporting values of median fluorescence intensity (MFI) and concentration data.

Quantitative polymerase chain reaction assays of coronavirus disease 2019 swabs

Real time reverse transcriptase polymerase chain reaction (RT-PCR) for COVID-19 PCR swabs were obtained from amniotic fluid, umbilical cord, placental, umbilical cord blood, maternal blood and high vaginal swabs and breast milk (15, 16).

Placental sample preparation and examination

Placental specimens were fixed in formalin overnight before representative sections were obtained from the umbilical cord, placental membranes and placental disc. The tissue was subsequently processed and stained with hematoxylin and eosin for microscopic examination.

Statistical analysis

The OD of samples obtained by subtracting the background signals and normalizing the samples against a negative control to account for interpolate variations. Data analyses were performed using GraphPad Prism (GraphPad Software, version 7.0.0). To test for normality, the Shapiro-Wilk test was used. For demographics and clinical features, student's t-test was performed for quantitative data and chi-squared test was performed for nominal data. Unless otherwise stated, statistical significance is defined as p-values being less than 0.05.

Results

Clinical demographics of the gestational immunity for transfer cohort

Eight women with a diagnosis of laboratory confirmed COVID-19 during pregnancy participated in the study (**Supplementary Table 1**). Nine women with no antenatal COVID-19 were recruited as controls. On average, COVID-19 convalescent women were 30.3 ± 2.4 years old. Additional characteristics of the study participants and their infants are presented in **Table 1**. Of the 8 women, 6 had mild COVID-19, with the most common being dry cough, sore throat and malaise, with one having moderate COVID-19 and the remaining person being asymptomatic. None of them progressed toward hypoxia or exhibited signs of lower respiratory tract infection (LRTI) evidenced by chest X-ray abnormalities. Consequently, none of the subjects required intensive care unit admission, had oxygen requirements, or were intubated. Individual clinical demographics of the convalescent women are listed in **Supplementary Table 2**. Extensive testing of placental and umbilical cord samples as well as infant throat swabs all produced negative PCR results for COVID-19 (detailed reporting of results in **Supplementary Table 3**).

The eight infants were all born full term with mean gestational age (SD) of 39 ± 2 weeks and had a mean birth weight (SD) of 3.517 ± 0.261 kg. There were no significant differences in terms of gestational age or birth anthropometry

TABLE 1 Demographic and clinical characteristics of the cohort.

Maternal characteristics	Cases ($n = 8$)	Controls ($n = 9$)	*P*-values
Age (average; years) (standard deviation, SD)	30.3 ± 2.4	32.1 ± 2.5	0.2963
Race/ethnicity			
Chinese	1	6	0.1084
Malay	3	2	
Indian	2	1	
Caucasian	2	0	
BMI (SD)	27 ± 2.2	25.7 ± 1.5	0.3875
Clinical features			
No past medical history	7	8	
Other co-morbidities if any			
– Hepatitis C carrier	1	2	
– Gestational diabetes mellitus (GDM)	0	1	
– Smoking	1	1	
– Pre-eclampsia	0	0	
Antenatal fetal anomaly scan	No abnormalities detected	No abnormalities detected	
Occurrence of mastitis during the study period	0	0	
Timing of COVID-19 infection			
First trimester	2	–	
Second trimester	2	–	
Third trimester	4	–	
Severity			
Symptomatic	6 Mild, 1 moderate	–	
Asymptomatic	1	–	
Infant characteristics	**Cases ($n = 8$)**	**Controls ($n = 9$)**	***P*-values**
Female	6	6	> 0.9999
Gestational age when born (weeks) (SD)	39 ± 2	39 ± 1	0.1760
Growth at birth			
Birth weight (kg) (SD)	3.517 ± 0.261	3.108 ± 0.376	0.0710
Length (cm) (SD)	50.5 ± 1.2	50.6 ± 1.3	0.4972
Occipital frontal circumference (cm) (SD)	34.5 ± 0.7	33.3 ± 0.7	**0.0416**
Breastfeeding status			
Exclusive	6	9	
Mixed feeding	2	0	

$P < 0.05$

between cases and controls except for occipital frontal circumference. A control infant CT09 was born premature at 30 weeks with a birth weight of 1.642 kg and occipital frontal circumference of 28 cm, which led to this difference. One infant required neonatal intensive care unit stay for 2 days for transient tachypnea of the newborn and was given supplemental oxygen therapy. All infants were breastfed up to 3 months, two of whom were supplemented with formula milk during the same period. Individual clinical demographics of the infants born to convalescent women are listed in **Supplementary Table 4**.

It remains controversial whether SARS-CoV-2 can be vertically transmitted and if so, what damage it might cause to the developing child and the maternal-fetal interface i.e., the placenta, especially in continued pregnancy post convalescence from antenatal COVID-19 (17–19). To address that question, histological examination of placental samples was done to determine if there were notable pathologies or abnormalities. While all 8 samples did not show the presence of SARS-CoV-2, three placentae (CS02, CS03, CS07) showed signs of acute inflammation (**Figure 1**). Neutrophils were seen in the subchorionic fibrin, indicating subchorionitis. There was no

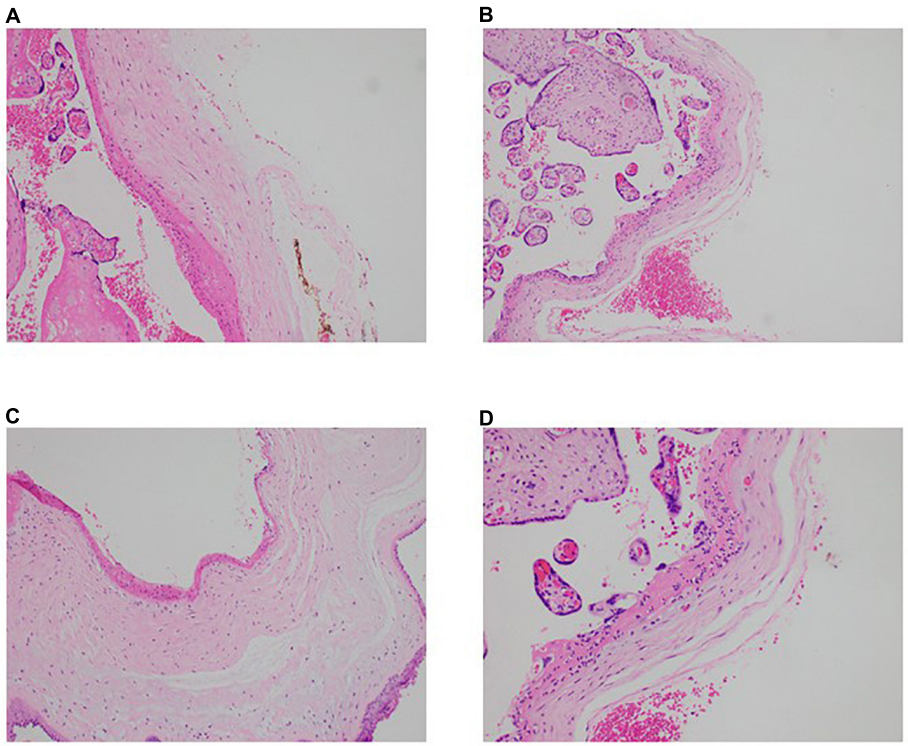

FIGURE 1
Neutrophilic infiltration present in the placentae of convalescent mothers. Microscopic analysis shows a neutrophilic infiltrate in the subchorionic region of the placentae of three patients—**(A)** patient CS2, H&E × 100, **(B)** patient CS3, H&E ×100, **(C)** patient CS7, H&E × 100. **(D)** Higher power view of the inflammatory process (patient CS3, H&E × 200).

spread of the neutrophils into the chorion or amnion. A mild corresponding fetal inflammatory response was also seen in the umbilical arteries and veins of one of the placentas (CS03). The placenta of CS05 had signs of maternal-fetal malperfusion, and this placenta was also hypoplastic at 389g at 38+0 weeks gestation). However, CS05 had concomitant gestational diabetes mellitus and the placental abnormalities in this subject was considered attributable to the diabetes.

Convalescent mothers carry severe acute respiratory syndrome coronavirus 2 specific antibodies in their blood

We first confirmed the presence of IgG antibodies targeting SARS-CoV-2 spike protein (**Figure 2A**) and spike protein RBD (**Figure 2B**) in all mothers' plasma 1-month post-partum. Plasma IgA antibodies targeting the same antigens were expressed more variably; they were detected in ∼50% of the convalescent mothers (**Figures 2C,D**).

Most convalescent maternal plasma were able to neutralize SARS-CoV-2 pseudoviruses, albeit to varying extents (**Figure 3**). Interestingly, plasma from one mother—CS04—showed no detectable pseudovirus neutralizing ability despite spike- and RBD-specific IgG antibodies being present and comparable to other convalescent mothers. These antibodies are likely to confer some degree of protection against SARS-CoV-2 infections. It is worth noting that neutralization capacity did not correlate with the length of time between disease resolution and point of sampling. In other words, the women who were infected at an earlier stage of pregnancy did not necessarily produce lower titers as one would expect due to antibody decay and contraction of the humoral response.

Four immunodominant epitopes S14P5, S20P2, S21P2, and N4P5 were previously identified to be associated with disease severity in adults (age 41 ± 13, years ± standard deviation) (12). Importantly, antibodies against S14P5 and S21P2 epitopes were able to neutralize SARS-CoV-2-infection (12, 13, 20). Hence, we evaluated the presence of antibodies against the four immunodominant epitopes in our cohort. A significant increase in the levels of IgG against S21P2 (Mann Whitney, two tailed, $p = 0.0205$) was observed in the plasma of convalescent mothers compared to controls (**Supplementary Figure 1C**). No significant difference in the levels of IgG against the other three immunodominant

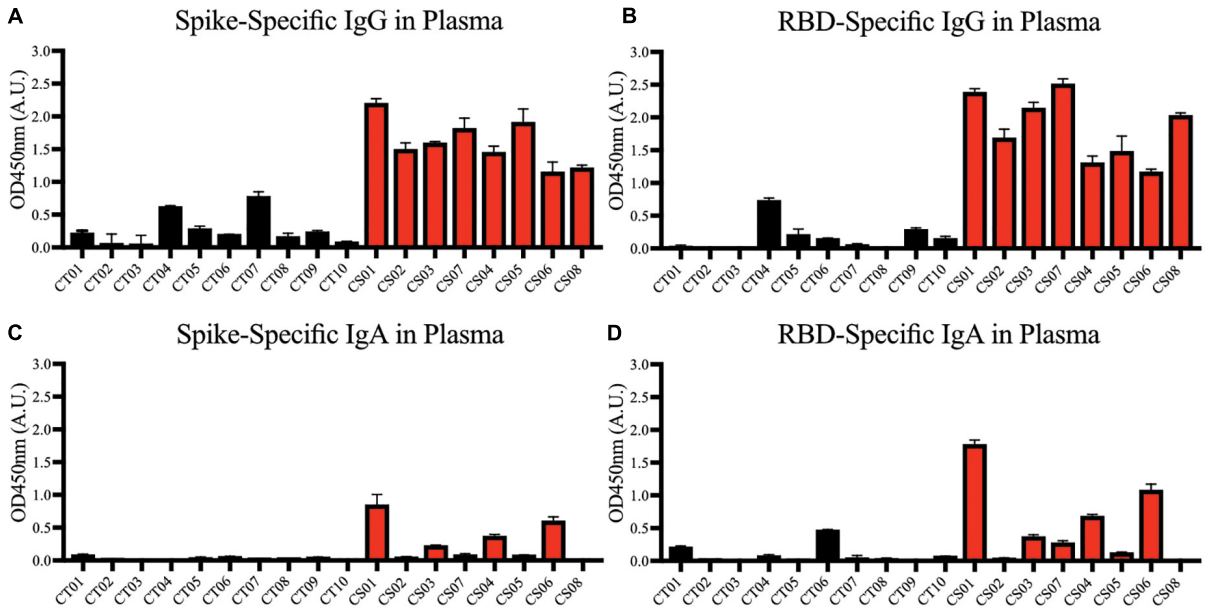

FIGURE 2
Spike and RBD-specific IgG and IgA present in maternal plasma of convalescent mothers. Using a protein-based ELISA, maternal plasma from control (CT01- CT10) and convalescent (CS01-CS07) mothers obtained 1-month post-partum were used for the detection of spike **(A,C)** and RBD **(B,D)** specific IgG and IgA antibodies. The bar graphs represent the normalized average signals of antibodies binding to the respective proteins. Convalescent mothers (CS samples) are arranged in ascending order according to their time from COVID diagnosis to delivery.

epitopes in the plasma of convalescent mothers compared to controls was found (**Supplementary Figures 1A,B,D**). The IgA levels against the four immunodominant epitopes in convalescent mothers were mostly below the limit of detection. Hence differences in IgA levels in the plasma between both groups cannot be accurately determined (**Supplementary Figure 2**).

Severe acute respiratory syndrome coronavirus 2 specific antibodies transferred *in-utero* to the child wanes over 6 months

An important component of passive immunity is the suite of antibodies transferred across the placenta from mother to child. As shown in **Figures 4A,B**, compared to the mothers, SARS-CoV-2 specific IgG antibodies were detected in the infants' sera at comparable or higher levels at birth, showing passive immunity to be successfully transferred via the placenta. We observed a steady decrease in plasma IgG levels in infants from birth to 6 months of age, where the signal approached the lower detection limit. The transplacental transfer ratios for spike-specific IgG antibodies ranged from 0.61 to 1.47, and RBD-specific IgG ranged from 0.73 to 1.46 (**Figures 4C,D**), in alignment with previous COVID-19 studies and other types of pathogens (21–23).

Severe acute respiratory syndrome coronavirus 2 specific antibodies are not appreciably secreted into convalescent milk

Apart from transplacental transfer of antibodies, another important mechanism of transmitting passive immunity to the child is via BM. Hence, we sought to measure virus-specific antibody titers in milk of mothers convalescent from antenatal COVID-19. Generally, minimal SARS-CoV-2 spike- and RBD-specific IgG and IgA antibodies were present in milk at 0–3 months postpartum (**Figure 5**). The same was observed for the epitope-specific antibodies (**Supplementary Figure 3**).

We noted a singular case, CS04, who produced S21P2-specific IgA responses in plasma and milk that were significantly higher than others, with no decrease observed up to 3 months postpartum (**Supplementary Figures 2C, 3C**). Notably, CS04 suffers from chronic high-viral load hepatitis C virus (HCV) infection, where the virus is of genotype GT3a. CS04 did not have unusually high serum IgA (**Figures 2C,D**). Hence, we asked the question whether HCV genotype GT3a encodes S21P2-like antigens that could induce cross-reactive antibody production. Initial Protein BLAST revealed no significant overlap between S21P2 and HCV proteins, including those encoded by GT3a (**Supplementary Data 1**). We then compared CS04 against CT10, a control mother who was COVID-19-negative but was HCV genotype GT3a-positive with comparable viral loads.

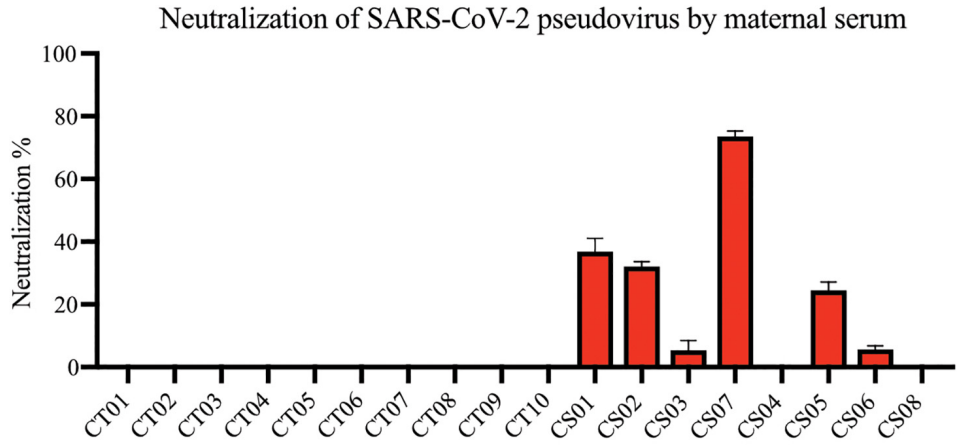

FIGURE 3
Neutralization of SARS-CoV-2 pseudovirus using maternal plasma. Plasma from control (n = 10) and convalescent mothers (n = 7). Bar graphs represent the average percentage neutralization. Convalescent mothers (CS samples) are arranged in ascending order according to their time from COVID diagnosis to delivery.

Unlike CS04, CT10 did not have any pre-existing antibodies against S21P2 (**Supplementary Figures 2C, 3C**). This suggests that the high titers of S21P2-specific IgA in CS04 were not due to cross-reactive IgA-mediated immunity against HCV. It might be that carriage of HCV results in a stronger mucosa-directed anti-viral response marked by higher titers of anti-S21P2 IgA. Nonetheless, we acknowledge the key limitation of small sample size, given the rarity of co-infections by SARS-CoV-2 and HCV in pregnant women; larger studies will be required to clarify interactions or lack thereof between the two viruses.

Persistence of pro-inflammatory cytokines in convalescent mothers

To gain insight on long-term perturbations in the maternal immune system post-COVID 19, we quantified levels of cytokines with a 41-plex microbead-based immunoassay. Using plasma samples of six control and seven convalescent mothers at 1-month post-partum, we could detect 33 out of 41 cytokines (**Figure 6**). As far as the assayed cytokines were concerned, both healthy and convalescent mothers had largely similar signatures. Most of the signals for the detected cytokines were not distributed normally, likely due to the fact that they clustered near the lower limit of detection. Notwithstanding, five cytokines, namely interleukin-17RA (IL-17RA), eotaxin, interferon-gamma produced protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1β (MIP-1β) exhibited Gaussian distributions and were likely more robust biomarkers. Hence, we focused on these cytokines for further analysis. In order to detect cytokines with increased expression in convalescent mothers, we utilized a statistical threshold of $p < 0.1$ due to small sample size and low power in this study. We found that pro-inflammatory cytokines IL-17RA ($p = 0.02$) and MCP-1 ($p = 0.0766$) were elevated in convalescent mothers than healthy controls, suggestive of a prolonged inflammatory state long after COVID resolution (**Figures 6B,C**).

Severe acute respiratory syndrome coronavirus 2 specific antibody titers in convalescent milk are boosted with a single dose of Pfizer/BioNTech's BNT162b2 mRNA vaccine

Given the observation that IgG transferred via the placenta only lasted for about 6 months in the infant, and the scarcity of SARS-CoV-2 specific antibodies in convalescent milk, we investigated alternative methods of passive immunity to protect infants from potential SARS-CoV-2 infection. Three convalescent mothers from our original cohort who were still nursing were given one dose of the BNT162b2 (Pfizer/BioNTech) mRNA vaccine. BM was taken at 4 time points, namely before vaccination, 3 days post-vaccination (PV), 7 days PV and 30 days PV. SARS-CoV-2 specific antibodies were not detectable in milk before vaccination. At 7–30 days PV, both SARS-CoV-2 specific IgG and IgA were generally detected at much higher levels (**Figure 7**), although CS08 did not secrete detectable levels of RBD-specific IgA into her milk (**Figure 7B**).

Discussion

In this study, we show that women who had acquired COVID-19 during pregnancy have high levels of circulating

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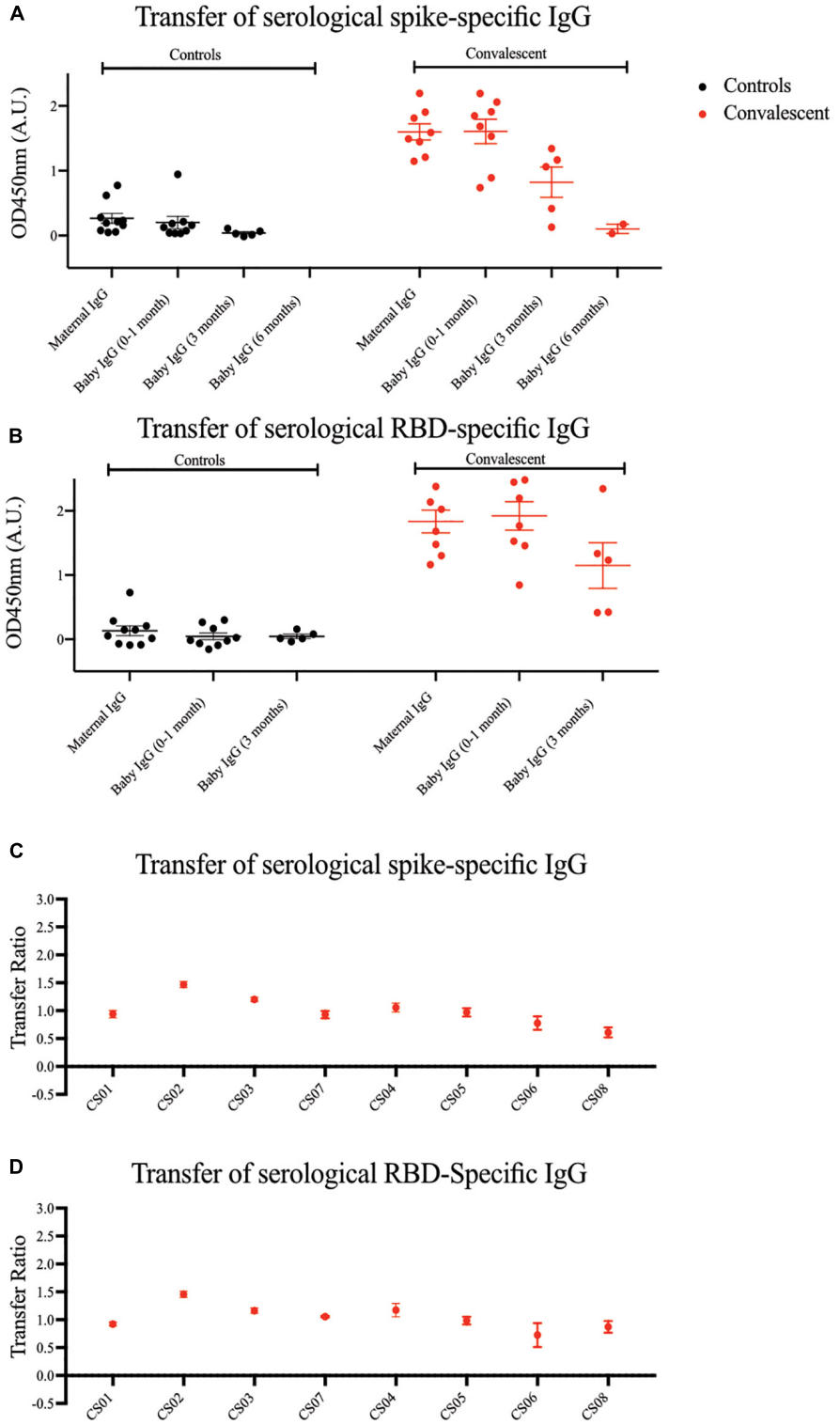

FIGURE 4
Spike and RBD-specific IgG are passively transferred from convalescent mothers to infants. **(A)** Spike and **(B)** RBD-specific IgG from maternal plasma 1 month post-partum and at three time points from babies' plasma was determined using ELISA. Transfer ratio at the 1 month timepoint of **(C)** spike and **(D)** RBD-specific IgG from mother to infant was calculated by taking the averaged IgG signal of baby's over the mother's IgG. Convalescent mothers (CS samples) are arranged in ascending order according to their time from COVID diagnosis to delivery.

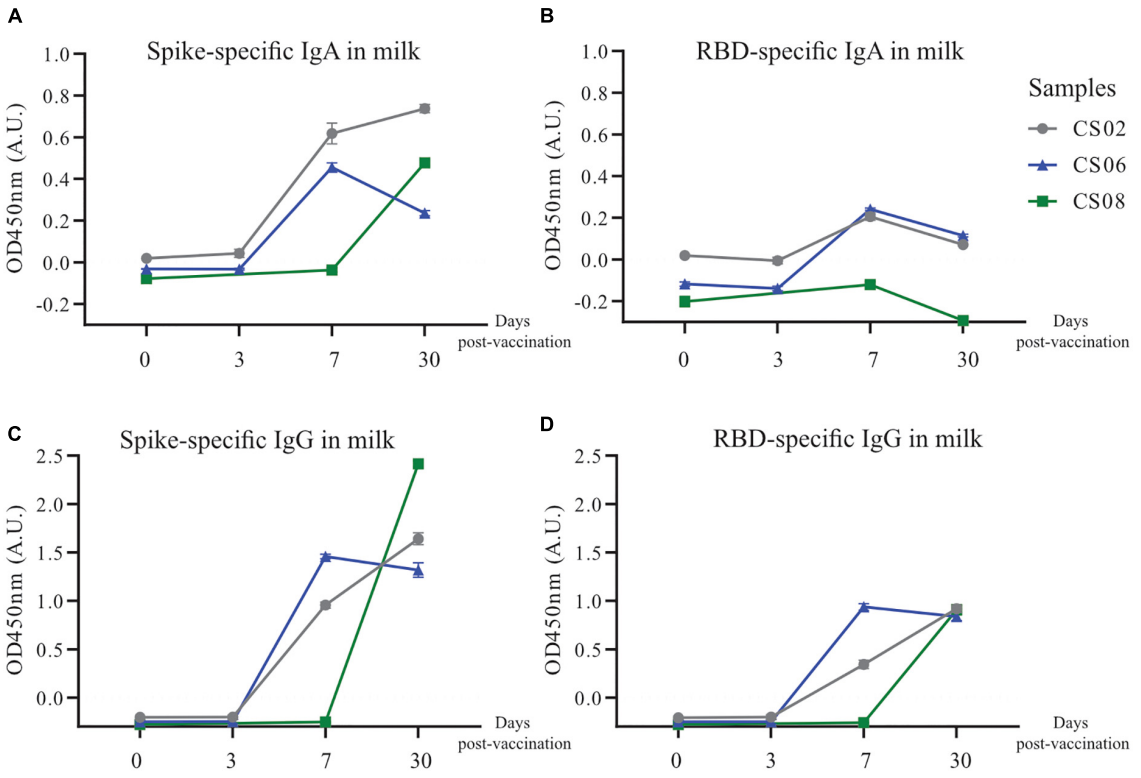

FIGURE 5
Spike and RBD-specific IgA present in maternal breast milk of convalescent mothers. Using a protein-based ELISA, maternal breast milk from control (CT01- CT10) and convalescent (CS01-CS08) mothers obtained at two time points 0–1 months and 2–3 months post-partum were used for the detection of spike and RBD-specific IgG and IgA antibodies. Breast milk was screened for **(A)** spike-specific IgG, **(B)** RBD-specific IgG, **(C)** spike-specific IgA, and **(D)** RBD-specific IgA. The bar graphs represent the normalized average signals of antibodies binding to the respective proteins. Convalescent mothers (CS samples) are arranged in ascending order according to their time from COVID diagnosis to delivery.

virus-specific IgG in their blood relative to control mothers. Furthermore, spike- and RBD-specific IgG levels in maternal circulation were maintained at relatively high levels regardless of gestation when SARS-CoV-2 infection was acquired in pregnancy; virus-specific IgG could be detected in infants at birth when mothers had acquired antenatal COVID-19 as early as 10 weeks of gestation. However, circulating spike- and RBD-specific IgG levels in infants born to convalescent COVID-19 mothers declined to negligible levels by 3 months of life. These findings are all consistent with the known patterns of acute gestational infections and passive immunity.

While virus-specific IgG was robustly expressed in convalescent plasma, virus-specific IgA levels in the blood were far more variable. IgA is a marker of acute infection and wanes just as IgG expression starts to pick up during disease resolution (24, 25). Hence, the expectation is that the shorter the interval between virus clearance and the point of sampling, the higher the IgA titers measured at the time of sample collection. We did not see such a clear pattern, which may be due to our small sample size and other factors related to host genetics and the environment that may modulate the plasma IgA response.

It is worth noting that, of the placental samples examined, CS02, CS03, and CS07 showed up abnormal findings indicative of acute inflammation, more than 50 days after their nasopharyngeal swab tested negative by PCR. These findings are broadly consistent with previous studies documenting acute inflammatory pathology, maternal vascular malperfusion, fibrinoid changes, and macrophage infiltration (26, 27). Most of the literature on placental pathology in the context of COVID-19 in pregnancy look mainly at acute perinatal infection—trophoblast necrosis and chronic histiocytic intervillositis are common in cases of vertical transmission, whereas materno-fetal malperfusion and villitis are common where there is no vertical transmission. To our knowledge, there are no reports of placental pathology in women convalescent from antenatal COVID-19 at the point of delivery. Interestingly, the four convalescent mothers whose placentae showed abnormal characteristics also showed the lowest titers of virus-specific IgA in their blood. Plasma IgA is predominantly monomeric and capable of engaging FcαRI (also known as CD89) on myeloid cells, presumably to trigger the first line of anti-viral defense. One may speculate that placental granulocytes perform a largely

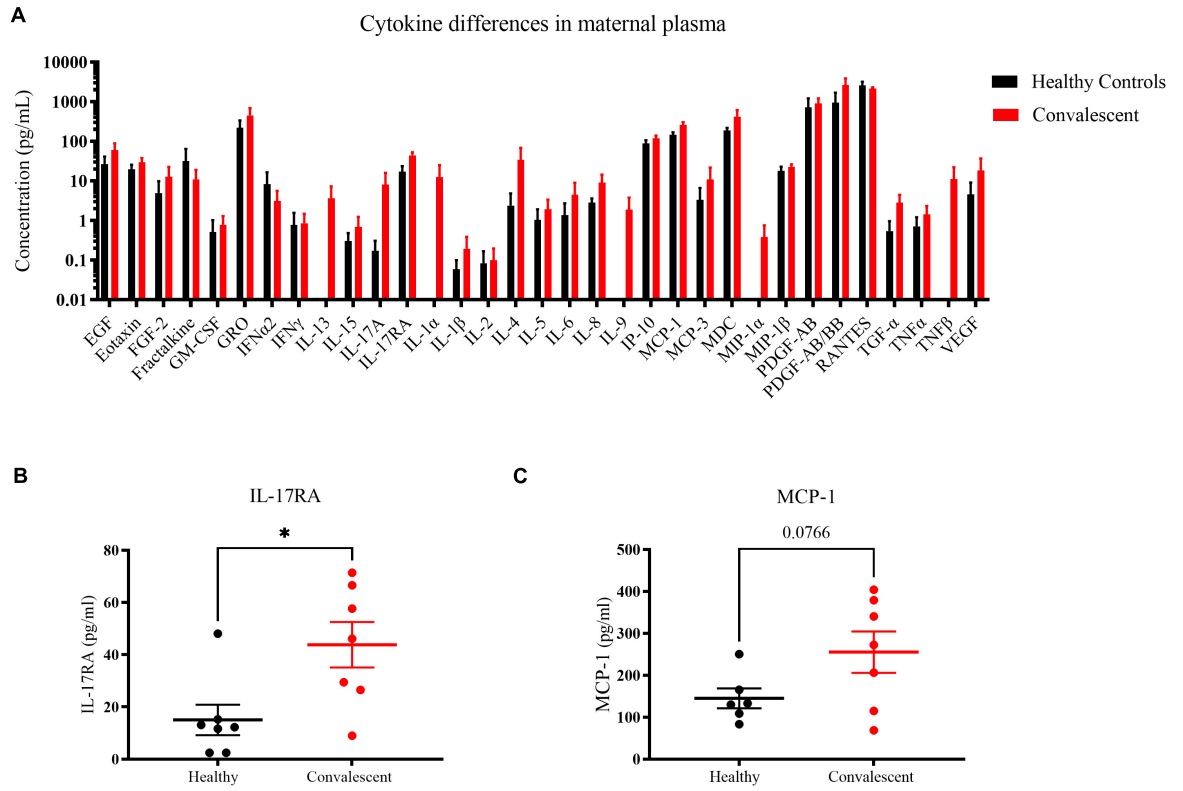

FIGURE 6
Cytokine profile differences in healthy and convalescent mothers' plasma. Using 1-month post-partum plasma (n = 6 for controls and n = 7 for cases), cytokines were evaluated using the 41-plex Luminex™ assay. **(A)** A representation of all cytokines that were detected, **(B)** IL-17RA levels and **(C)** MCP-1. Convalescent mothers are represented in red while healthy controls are represented in black. Bar graphs represent the average log(concentration) from the samples from the respective groups in A while the measured concentrations were used for **(B,C)**. *$p < 0.05$.

sentinel role to prevent compromise of the placental barrier, and in the absence of high amounts of plasma IgA indicative of active infection, they might be continually and preferentially mobilized to the placenta leading to low levels in the circulation (28).

Inefficient transplacental antibody transfer has been previously reported in a small cohort study of COVID-19 mothers (22). However, we did not see this particular effect in our cohort, even in the mothers who acquired COVID-19 in the third trimester and whose placentae exhibited signs of inflammation and potential tissue damage. These discrepancies might be due to differences in severity stratification (ratio of asymptomatic: mild: moderate: severe was 6:9:3:4 in Atyeo et al. vs. 1:6:1:0 in our cohort) and symptom onset to delivery (median of 30.5 days in Atyeo et al. vs. 98.5 days in our cohort). Also, transplacental transfer occurs most efficiently late into the third trimester, and so infections occurring and resolving earlier in the first and second trimesters are less likely to impact transfer (29).

In our study, we noted significant elevation in IL-17RA and MCP-1 levels in convalescent mothers relative to controls (**Figures 6B,C**). IL-17RA is a soluble receptor of IL-17A, thereby preventing the latter's association with cell surface receptors. IL-17A has been implicated in SARS-CoV-2 pathogenesis with elevated peripheral IL-17 levels and Th17 infiltration in the lungs of COVID patients (30, 31). The persistence of IL-17A together with other inflammatory cytokines such as IL-12p70, stem cell factor, and IL-1β was discovered in patients up to 180 days post-infection (32). In agreement with our findings, COVID-19 patients were found to have increased IL-17RA levels during the acute phase, especially in those with milder symptoms (33). Hence, enhanced IL-17RA may have a protective role in limiting the downstream effects of IL-17; elevated IL-17RA levels may be a prognostic marker of mild disease in pregnant and lactating women. MCP-1 is the other cytokine that was slightly elevated in convalescent mothers relative to control mothers. It is produced by monocytes and macrophages to regulate the migration and infiltration of natural killer cells, monocytes, and T cells (34). Greater MCP-1 expression characterizes patients with mild COVID-19, suggestive of potential roles in mitigating severity (35). However, we note that the lack of severe cases in our cohort limits us from investigating whether high IL-17RA and MCP-1 levels are true predictors of mild disease.

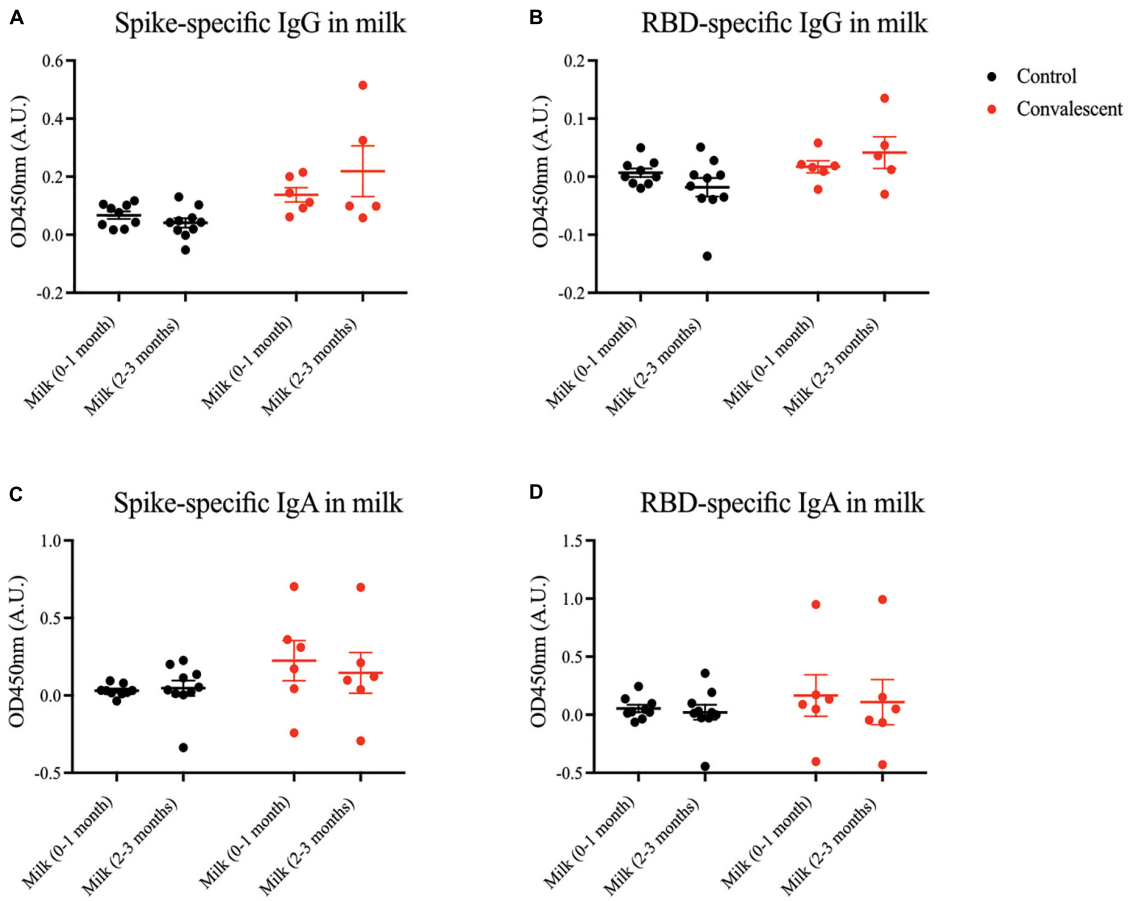

FIGURE 7
Increased secretion and production of spike-specific IgA and IgG antibodies post-vaccination in convalescent mothers. Breast milk from three nursing convalescent mothers were collected at 4 time points, prior to vaccination (0), 3 days post-vaccination, 7 days post-vaccination, and 30 days post-vaccination for a **(A,C)** spike and **(B,D)** RBD protein-based IgA and IgG ELISA assay. The *x*-axis refers to the number of days post-vaccination.

In breast milk, low levels of spike-specific IgG were secreted into 2 out of 8 convalescent women's BM. This is consistent with the existing dogma that IgG is rarely released into milk; the dominant isotype is IgA. Even so, virus-specific IgA was only present in a subset of convalescent mothers' milk samples. This is somewhat expected as IgA levels are not maintained throughout infection and have been found to be lowest on the outset in mild cases, where most of the subjects in our cohort fall under (24).

We further delineated the humoral response to linear B cell epitopes that have been previously described to be immunodominant. There are certain advantages in investigating these epitopes instead of conformational ones—(1) the system is considerably cheaper to set up for further validation studies, and (2) it is easier to prime cells with peptides which can be synthesized conveniently and do not require complex purification and refolding protocols. We found that most linear epitopes were generally not well-recognized by antibodies present in sera and milk from convalescent mothers. This might be due to the fact that long periods of time had passed between COVID-19 recovery and sampling points; the antibody levels waned, similar to that for other infections. One notable exception is S21P2, where epitope-specific IgG was significantly elevated in convalescent plasma compared to controls. In particular, CS04 produced extremely high levels of S21P2-specific IgA in the BM by mechanisms unknown to us, although preliminary analysis has excluded HCV antigen cross-reactivity as one possible factor. Nonetheless, much of these signals were non-specific and could also be detected in controls who had non-SARS-CoV-2 coronavirus infection or non-coronavirus antenatal URTI.

Given that convalescent sera do contain high levels of spike- and RBD-specific IgG and yet exhibit low, variable neutralization capacities, we infer that most of the IgG are poorly functional in terms of blocking receptor binding, which is consistent with a previous report showing low neutralizing

antibody titers in mild cases (36). The current design of the mRNA vaccines utilizes the 2P mutated spike to stabilize its pre-fusion conformation. As these convalescent women had not received the vaccine prior to infection, their immune systems likely encountered virions that had a mix of pre-fusion and post-fusion trimers, the latter having evolved to perhaps fulfill immunoevasive functions (37). The consequence for these individuals is the production of weakly neutralizing antibodies that primarily target the post-fusion conformational epitopes. Had the infected individuals produced strongly neutralizing antibodies, those antibodies would have been predominantly raised against the pre-fusion conformation. Given that a single dose of the Pfizer/BioNTech mRNA vaccine was sufficient to boost virus-specific IgG and IgA responses in mothers who had long convalesced from natural SARS-CoV-2 infection, we speculate that the booster-elicited antibodies are directed against both the pre- and post-fusion forms; recall responses are activated in cells recognizing the post-fusion spike, and primary responses are generated in response to the vaccine-encoded pre-fusion spike. Together, these findings underscore the importance of receiving the vaccine even after recovery from natural infection. Additionally, given the lack of safety and efficacy data in administering vaccines to infants, breastfeeding after vaccination could be a viable alternative to conferring some form of mucosal immunity to the vulnerable children, after the loss of transplacentally transferred antibodies at 3 months of age.

A key limitation of our study is the cohort size. Most of our samples were collected early on in the pandemic before Singapore introduced a slew of effective public health measures to stymie the spread of the virus, including travel restrictions, social distancing, and a lockdown. Due to this unique situation, relatively few people were infected in the first wave from April to August 2020. Limited community transmission between August 2020 and August 2021 prevented further subject recruitment and sample collection.

In the context of the present situation, we note two caveats, namely the current dominance of Delta and Omicron over early pandemic variants and the high rate of vaccine uptake by the resident population (∼92% as of 31st March 2022). These two factors preclude meaningful head-to-head comparisons of more recent studies against our cohort in the GIFT study where immunologically naïve individuals were infected with variants possessing lower immunoevasive abilities. Notwithstanding these differences, our studies provide insights into the pathogenesis of COVID-19 in the under-studied demographics of pregnant and lactating women as well as infants born to them.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the National Healthcare Group Institutional Review Board (Gestational Immunity For Transfer GIFT: DSRB Reference Number: 2020/00483). Written informed consent was obtained from all subjects (and where applicable, parents), and the study was conducted in accordance with the Helsinki Declaration. The study protocol was registered at clinicaltrials.gov (NCT04802278). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JL and YZ contributed to conceptualization of the study. JL, YZ, and LW were in charge of project administration. YG, JL, JT, BS, RG, DL, and LW performed the experiments. YG, JL, JT, MN, DL, YZ, and LW analyzed the data. JL and LL obtained funding. JL, PM, and ZA provided resources for this study. PM, ZA, LL, LS, and LW supervised the study. YG, JL, JT, MN, YZ, and LW prepared the original manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.949756/full#supplementary-material

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Somatosensory abnormalities after infection with SARS-CoV-2 – A prospective case-control study in children and adolescents

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Background: Long-term neurological complaints after SARS-CoV-2 infection occur in 4–66% of children and adolescents. Controlled studies on the integrity of the peripheral nerve system are scarce. Therefore, we examined the somatosensory function in children and adolescents after SARS-CoV-2 infection in a case-control study compared with age-matched individuals.

Materials and Methods: Eighty-one subjects after SARS-CoV-2 infection (n = 44 female, 11.4 ± 3.5 years, n = 75 SARS-CoV-2 seropositive, n = 6 PCR positive during infection and SARS-CoV-2 seronegative at the time point of study inclusion, n = 47 asymptomatic infection) were compared to 38 controls without SARS-CoV-2 infection (26 female, 10.3 ± 3.4 years, n = 15 with other infection within last 6 months). After standardised interviews and neurological examinations, large fibre (tactile and vibration detection thresholds) and small fibre (cold and warm detection thresholds, paradoxical heat sensation) functions were assessed on both feet following a validated protocol. After z-transformation of all values, all participants were compared to published reference values regarding the number of abnormal results. Additionally, the mean for all sensory parameters values of both study groups were compared to an ideal healthy population (with z-value 0 ± 1), as well as with each other, as previously described. Statistical analyses: t-test, Chi-squared test, and binominal test.

Findings: None of the controls, but 27 of the 81 patients (33%, $p < 0.001$) reported persistent complaints 2.7 ± 1.9 (0.8–8.5) months after SARS-CoV-2 infection, most often reduced exercise capacity (16%), fatigue (13%), pain (9%), or paraesthesia (6%). Reflex deficits or paresis were missing, but somatosensory profiles showed significantly increased detection thresholds for thermal (especially warm) and vibration stimuli compared to controls. Approximately 36% of the patients after SARS-CoV-2, but none of the controls revealed an abnormal sensory loss in at least one parameter ($p < 0.01$).

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Sensory loss was characterised in 26% by large and 12% by small fibre dysfunction, the latter appearing more frequently in children with prior symptomatic SARS-CoV-2 infection. Myalgia/paraesthesia was indicative of somatosensory dysfunction. In all eight re-examined children, the nerve function recovered after 2–4 months.

Interpretation: This study provides evidence that in a subgroup of children and adolescents previously infected with SARS-CoV-2, regardless of their complaints, the function of large or small nerve fibres is presumably reversibly impaired.

KEYWORDS

COVID-19, SARS-CoV-2, quantitative sensory testing, somatosensory function, children, adolescents

Introduction

Persisting complaints >4 weeks after SARS-CoV-2 infection, have been reported in 4–66% of affected children and adolescents despite a mostly mild course of infection (1–3). However, most data are based only on patient-reported outcomes, and the underlying pathomechanisms of most reported complaints remain unclear, especially whether they are correlated to an actual organ damage. Remarkably, many children complaining of dyspnoea do not suffer from persistent deterioration of respiratory function (4).

Although neurological complications are very rare in children and adolescents, changes in both the central and peripheral nervous system have been described (5–8), similar to other viral respiratory infections (9, 10). Some of the reported complaints after SARS-CoV-2 infection might indicate an affection of the peripheral or central nervous system, for example, smell and taste disorder, persisting paraesthesia, myalgia, limb pain, and headache, which might be related to an affection of the somatosensory system (11). Different molecular mechanisms are discussed, that may be involved in the development of neurological complaints and pain (12). This seems to be a multifactorial pathophysiology. One possible explanation could be the influence of SARS-CoV-2 on the angiotensin-converting enzyme 2 and renin-angiotensin system (ACE2/RAS). SARS-CoV-2 binds to the ACE2 receptor, which is also expressed in neurons and glial cells and can thus cause damage to sensory neurons. In addition, there are processes such as macrophage activation and an abnormal production of cytokines as well as autoantibodies, which arise through molecular mimicry (12, 13). The cytokine storm during severe COVID-19 infection has also been discussed as a potential driving factor for the development of neuropathies and could contribute to the development of chronic pain after the acute infection has resolved (14). These processes can cause neuronal damage and thus lead to acute and chronic impairment of function or pain (12). Some case reports have descried transient sensory loss (hypoalgesia) in adults several months after COVID-19 (15). Preclinical models indicate that SARS-CoV-2 spike protein subverts pronociceptive signalling including vascular endothelial growth factor-A and neuropilin-1 receptor (16).

To the best of our knowledge, no studies have analysed the somatosensory function in children with and without persisting complaints after infection with SARS-CoV-2.

Therefore, we compared the somatosensory function between children and adolescents with and without previously confirmed SARS-CoV-2 infection in a case-control study and in relation to persistent complaints. We performed quantitative sensory testing (QST), a validated and non-invasive approach to investigate the somatosensory function, including both small and large nerve fibres and the corresponding central pathways (17–19), according to the internationally accepted protocol of the German Research Network for Neuropathic Pain (DFNS e.V.) (17, 20–22). Its advantages include established reference data for children and adolescents older than 6 years (23, 24) and previous validation as a tool to assess neuropathy symptoms in children, for example, with type I diabetes mellitus, cerebral palsy or survivors of paediatric acute lymphoblastic leukaemia (25–27). Second, we analysed the possible influence of age and severity of previous infection on somatosensory performance and the type of long-term complaints 2–4 months after infection.

Materials and methods

The study was approved by the Ethical Committee of the Faculty of Medicine, Ruhr University Bochum, in July 2020 (Reg. no. 20-6927_1) and performed in accordance with

the Declaration of Helsinki. Participants were recruited from August 2020 to May 2021.

Participants

Children and adolescents (aged 6–18 years) suspected of having had SARS-CoV-2 infection and interested participants from a population-based cohort study of children screened for SARS-CoV-2 infection (CorKid – SARS-CoV-2 seroconversion in kids) were included in the study.

Participants had a history of asymptomatic or symptomatic infection [confirmed by a reverse transcriptase–polymerase chain reaction (PCR) and/or antinuclear SARS-CoV-2 IgM and IgG antibodies COI > 1.000]. In six cases of positive viral detection by PCR during the acute infection, no SARS-CoV-2 IgM and IgG antibodies were detectable at the time point of study inclusion. The control group consisted of children and adolescents without evidence of SARS-CoV-2 infection (negative PCR and antibodies) (**Supplementary Table 1**). Exclusion criteria in both groups were any neurological or psychiatric disorders, mental illnesses, or relevant chronic diseases. The pre-existing comorbidities listed in **Supplementary Table 1** are bronchial asthma, respiratory allergy and atopic dermatitis, which have no effect on peripheral nerve fibre function. In one case, familial Mediterranean fever was present.

Assessment procedure

A standardised interview included information about the date and severity of the prior infection, the results of the former PCR and/or SARS-CoV-2 antibody tests and a list of complaints (**Table 1**). The latter which were classified into four main categories: general complaints, neurological complaints, pain, and respiratory complaints. Participants with respiratory complaints such as dyspnoea underwent extensive lung function analyses, including body plethysmography and lung clearance index as recently described (4).

Quantitative sensory testing

Quantitative sensory testing was performed by a trained examiner (LE) who was blinded to the SARS-CoV-2 status of the participants at the time of QST examination and analysis. Thermal and mechanical detection thresholds were assessed at both dorsal feet according to the internationally accepted and recommended standardised protocol of the German research Network on Neuropathic pain (DFNS) and included the following six parameters: cold and warm detection thresholds (CDT, WDT), thermal sensory limen (TSL), paradoxical heat sensations (PHS), tactile mechanical detection threshold (MDT), and vibration detection threshold (VDT) (22). All procedures have been described in detail recently (23, 28). CDT, WDT, and TSL were tested using the thermal sensory testing device Q-Sense (MEDOC, Israel) with a contact area of 16 mm × 16 mm and cut-off temperatures of 16 and 45°C. Participants pressed a button once they felt cold or warm to determine the CDT and WDT (means of three measurements, respectively). TSL was assessed during a series of six alternating warm and cold stimuli, and participants pressed a button once they felt a temperature change. PHS was assessed by asking whether children perceived cold stimuli as warm during TSL. MDT was assessed using a standardised set of von Frey-Hairs (Marstock nervtest, Optihair 2, 0.25–512 mN). The threshold was determined as the geometric mean of a series of measurements of five ascending and descending stimuli. For the VDT assessment, we used a Rydel-Seiffert tuning fork (128 Hz). VDT was defined as the mean of three measurements.

Geber et al. (29) were able to demonstrate good test-retest and interobserver reliability [r = 0.86 (TRR; range 0.67–0.93) and r = 0.83 (IO-R; range 0.56–0.89)] for this assessment in a multi-centre study. Even over a period of 4 months, very good long-term reliability (intraclass correlation coefficients: 0.68–0.90) could be demonstrated for the parameters used in this work (30).

Statistics

All sensory parameters except PHS have been shown to be normally distributed (partly in log space) (28). Subsequently, the sensory results of all study participants were z-transformed (except PHS), with z-values < 0 representing loss of somatosensory function compared to the mean of the aged-matched reference dataset (23). Abnormal values are defined as values beyond the 95% confidence interval, i.e., z-values < −1.96 (indicating abnormal sensory loss, i.e., hypoesthesia) and z-values > 1.96 (indicating abnormal sensory gain, i.e., hyperaesthesia). The z-transformation normalises for age, gender, and tested body region, thus making the results comparable. PHS (absent in healthy individuals under normal conditions) is reported as original values as the arithmetic mean of occurrences of PHS (0–3). For each QST parameter, the value from the foot indicating a higher deviation from the cut-off value of the reference data (i.e., more sensory loss or more sensory gain, respectively) was used for further statistical analysis. QST results were defined as abnormal in case of any increased detection threshold, that is z-score of CDT, WDT, TSL, MDT, or VDT < −1.96, or if PHS was >0 (23).

Chi-squared tests were used to analyse the number of abnormally increased detections for each sensory parameter compared to the controls and to a theoretically assumed frequency of 2.5% for each loss and gain, resulting from

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TABLE 1 Long-term complaints newly emerged after SARS-CoV-2 infection.

	Post-SARS-CoV-2-group with current complaints			
	All	**Symptomatic infection (n = 35)**	**Asymptomatic infection (n = 47)**	***P* value**
At least one complaint, No. (%)	**27** (100)	**16** (46)	**11** (23)	**0.033**
General complaints, No. (%)	**19** (70)	**12** (75)	**7** (64)	**0.04**
Reduced physical capacity, No. (%)	13 (48)	9 (56)	4 (36)	0.035
Fatigue, No. (%)	11 (41)	7 (44)	4 (36)	0.13
Sleep disorder, No. (%)	6 (22)	3 (19)	3 (27)	0.71
Mental complaints, No. (%)	4 (15)	3 (19)	1 (9)	0.18
Cold feet, No. (%)	5 (19)	5 (31)	0 (0)	0.007
Skin alterations, No. (%)	1 (4)	1 (6)	0 (0)	0.030
Potentially neurological symptoms, No. (%)	**19** (70)	**12** (75)	**7** (64)	**0.04**
Smell/Taste dysfunction, No. (%)	9 (33)	6 (38)	3 (27)	0.12
Tingling paraesthesia, No. (%)	5 (19)	5 (31)	0 (0)	0.007
Vertigo, No. (%)	5 (19)	2 (13)	3 (27)	0.90
Muscle weakness, No. (%)	1 (4)	1 (6)	0 (0)	0.30
Dysphagia, No. (%)	1 (4)	1 (6)	0 (0)	0.30
Pain, No. (%)	**7** (26)	**5** (31)	**2** (18)	**0.11**
Headache, No. (%)	6 (22)	4 (25)	2 (18)	0.22
Myalgia, No. (%)	5 (19)	5 (31)	0 (0)	0.007
Joint pain, No. (%)	2 (7)	2 (13)	0 (0)	0.12
Burning pain, diffuse, No. (%)	1 (4)	1 (6)	0 (0)	0.30
Respiratory complaints, No. (%)	**12** (44)	**9** (56)	**3** (27)	**0.014**
Dyspnoea, No. (%)	9 (33)	7 (44)	2 (18)	0.024
Cough, No. (%)	3 (11)	2 (13)	1 (9)	0.39

Data are expressed as *No.* (%), unless specified otherwise. Bold values represent the categories of symptoms.

the definition of abnormal values, which is outside the 95% confidence interval of healthy subjects. Binominal test was used to analyse the frequencies of persistent complaints because of the small number in the study population. Unpaired t-tests were used to compare each QST parameter with the control group, as described in previous studies (28, 31). Additionally, QST z-values were compared to the expected values of an ideal healthy population with a mean z-value = 0 and a standard deviation = 1.

Results

Patient cohort

Study participants were recruited from 183 consecutive patients and their siblings from the Corona outpatient clinic and 32 subjects, who visited the CorKid study for follow-up (32) (for a STROBE-diagram, **Figure 1**). For the Corona outpatient clinic, families could call to register if any of their children had or were suspected of having a COVID-19 infection.

After exclusion due to age (<6 years), any comorbidity associated with peripheral neuropathy (like diabetic mellitus, cystic fibrosis) or declining neurological examinations, 81 children and adolescents with confirmed previous SARS-CoV-2 infection (age 11.4 ± 3.5 years, n = 67 tested with PCR, n = 14 tested with antibody test) were included ("SARS-CoV-2 group"). From the same cohort, 38 children and adolescent without history of previous SARS-CoV-2 infection and with negative antibody testing (age 10.3 ± 3.4 years) served as controls. Clinical and demographic data did not differ between the groups (**Supplementary Table 1**).

The assessment took place 2.8 ± 2.0 (0.8–8.8) months after PCR testing for SARS-CoV-2 or 2.6 ± 2.0 (0.7–6.3) months after symptoms of other viral infection. 42% of the participants in the SARS-CoV-2 group had a history of a symptomatic infection (n = 26 tested with PCR, n = 8 tested with antibody test) and 58% had asymptomatic infection (n = 41 tested with PCR, n = 6 tested with antibody test). In the control group, 40% reported other symptomatic viral infections in the 6 months prior to assessment. Symptoms during acute viral infections such as headache (24 vs. 11%), fever (25 vs. 13%), taste dysfunction (12 vs. 0%), and fatigue (35 vs. 21%) were reported more frequently in the SARS-CoV-2 group, although not statistically significant.

Persistent complaints after the acute viral infection

None of the controls, but 27 children and adolescents from the SARS-CoV-2 group (33%) reported at least one persistent complaint (**Table 1**) at the time of study enrolment. Sixteen

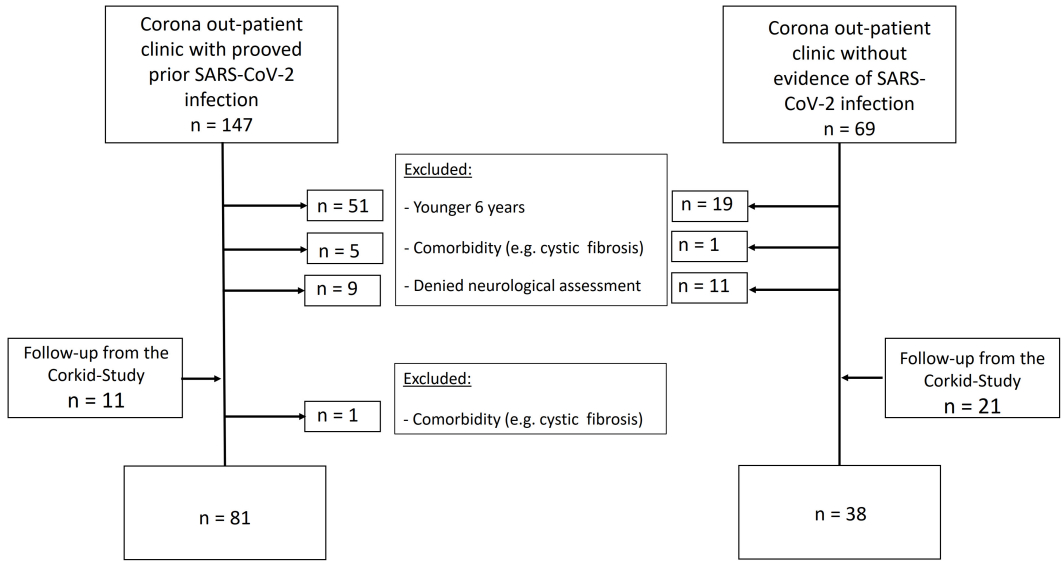

FIGURE 1
STROBE flowchart of included patients.

of these 27 children (59%) with a symptomatic SARS-CoV-2 infection reported at least one persisting symptom compared to 23.4% from the asymptomatic group ($P < 0.05$), resulting in a 2.3-fold higher probability of persistent complaints after a SARS-CoV-2 infection (CI 1.07–7.11). Nearly half of them reported decreased physical capacity, whereas ~70% described having any neurological symptoms (**Table 1**). Except for pain, all other symptoms occurred significantly more often in subjects with symptomatic SARS-CoV-2 infection than in those with an asymptomatic course.

Neither patients nor controls showed reflex deficits or paresis in the clinical examination. In 72 participants of the SARS-CoV-2 group, lung function was additionally assessed, and 13 (18%) of them presented with abnormal findings (symptomatic SARS-CoV-2 infection: $n = 6$, reported persistent complaints: $n = 5$). Lung function was assessed in 33 participants in the control group, and 24% of them showed abnormal findings (n.s.) (4).

Sensory profiles of quantitative sensory testing

The somatosensory parameters assessed during QST were, on average, within the normal range in both patients and controls (**Figure 2**). However, the detection thresholds for thermal and mechanical stimuli were higher in the SARS-CoV-2 group than in the controls, with significant differences in WDT ($P = 0.003$), TSL ($P = 0.02$), and VDT ($P = 0.02$) (**Figure 2**). The SARS-CoV-2 group deviated significantly from an ideal control group (mean ± SD: 0 ± 1 z-values) in each QST parameter, whereas the control group deviated significantly from the ideal only in TSL ($p = 0.012$). Among those in the SARS-CoV-2 group who had asymptomatic infection, all QST parameters were significantly decreased, except CDT, and among those with a symptomatic SARS-CoV-2 infection, all QST parameters significantly decreased except MDT.

There were no cases with abnormal sensory function (outside the reference values) in the control group. In contrast, in 36% of patients after SARS-CoV-2 infection, there was at least one abnormal QST parameter ($p < 0.001$). Abnormal values were found for thermal detection (WDT in 4%, CDT in 5%, and PHS in 6%) and even more frequently for mechanical detection (MDT in 10% and VDT in 17%; **Figure 3A**). Twenty-four patients (30%) had an abnormal result in only one QST parameter, five patients (6%) had ≥2 abnormal results. Sensory loss was observed in small fibre function in ten patients (12%) and in large fibre function in 21 patients (26%).

Patients with a history of symptomatic or asymptomatic SARS-CoV-2 infection had similar frequencies of at least one abnormally increased detection threshold (34 vs. 40%). However, within the subgroup with previous symptomatic SARS-CoV-2 infection a dysfunction of the small nerve fibres occurred significantly more frequently (21 vs. 4%; $P < 0.05$; **Figure 3B**).

Relation between somatosensory dysfunction and subjective complaints

Children and adolescents with persistent neurological complaints, particularly myalgia or paraesthesia, demonstrated

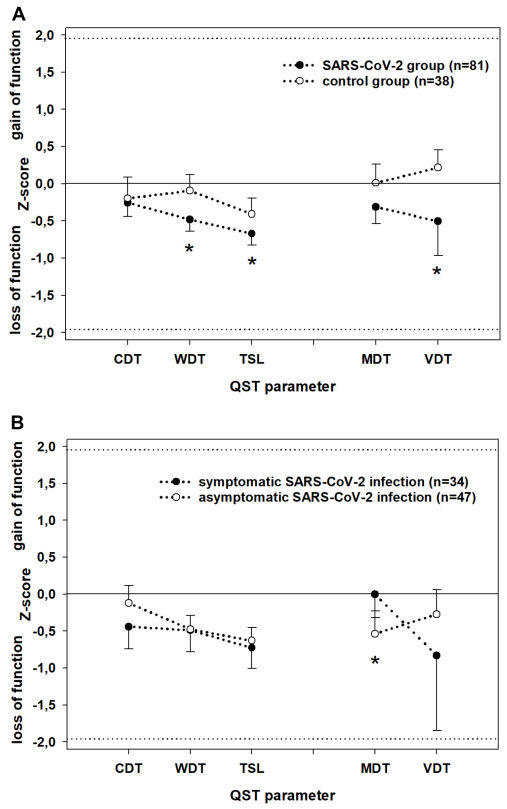

FIGURE 2
Detection thresholds at dorsal feet (z-score; mean ± SD) assessed by Quantitative Sensory Testing in **(A)** patients (black) vs. controls (white) and **(B)** patients with symptomatic (black) vs. patients with asymptomatic (white) SARS-CoV-2 infection. Parameters assessing the small (WDT; CDT; TSL) and large (VDT, MDT) fibre function have been separately linked by dotted lines for better visualisation. *Z*-values between −1.96 and 1.96: normal range of healthy subjects. *Z*-values > 0: gain of sensory function, *z*-values < 0: loss of sensory function. CDT, cold detection threshold; WDT, warm detection threshold; TSL, thermal sensory limen; MDT, mechanical detection threshold; VDT, vibration detection threshold. Significant differences ($P < 0.05$) are marked with *.

more frequently abnormal increased somatosensory detection thresholds and paradoxical heat sensations (myalgia/paraesthesia 17 vs. 0%) (**Table 1** and **Figure 4**). However, the occurrence of persistent long-COVID complaints was not associated with loss of sensory function (OR 1.3; CI 0.5–3.36).

Children with and without symptomatic infection had a similar prevalence of loss of sensory function (OR 1.29; CI 0.52–3.2).

The frequency of abnormal QST was similar in children with or without impaired lung function; of 13 out of 69 patients with SARS-CoV-2 infection and reduced lung function, five (39%) also had at least one abnormal detection threshold. Of the remaining 56 patients without abnormal lung parameters, 20 had an abnormal QST result (36%; $P = 0.85$).

A second follow-up QST assessment was performed in 26 patients of the SARS-CoV-2 group 4–12 months after infection (mean of 7.4 ± 2.4 months). The frequency of at least one abnormal QST parameter declined from 8 to 2 cases. Large fibre affection was recovered in all cases, whereas small fibre abnormalities persisted in two children, although only one of them reported ongoing complaints (leg pain and paraesthesia).

Discussion

To the best of our knowledge, this is the first study showing that one-third of children and adolescents experience abnormal somatosensory function for up to 7 months after asymptomatic or mild symptomatic SARS-CoV-2 infection in a tertiary Corona out-patient clinic. In particular, patients with persistent neurological complaints, such as myalgia or paraesthesia, also presented with isolated somatosensory abnormalities without signs of lesions in the motor system (e.g., paresis or diminished tendon reflex during the clinical examination). No child in the control group, even those with a previous viral infection, reported any complaint 3 months later or showed abnormal values in the clinical or QST examination. Somatosensory profiles of the group with a history of a SARS-CoV-2 infection revealed on a group level a significant sensory loss for thermal stimuli, especially warmth, abnormalities in the perception of temperature changes, and sensory loss for vibration stimuli. At the individual level, at least one QST parameter was abnormal in one-third of the patients ($P < 0.01$), mostly related to large fibre function with complete recovery 7 months after infection. A loss of small fibre function was less frequent (11%) and was still present 4 months later.

The spectrum of persistent complaints 3 months after infection with SARS-CoV-2 corresponds to previous reports (3, 33). In our investigation, patients who reported myalgia or paraesthesia [with a prevalence of 7%, previous reports ranging from approximately 3 to 60% (3)] more often presented with a loss of sensory function. Sensory loss is known to occur in nerve damage of other aetiologies, on the one hand, in pathophysiologically well-characterised entities such as diabetic peripheral neuropathy and chemotherapy-induced neuropathy, and on the other hand, in multifactorial syndromes like fibromyalgia (18, 25, 27, 34–36).

The somatosensory assessment (QST) used here is a functional test with high sensitivity for the detection of neuropathy (17, 18, 23, 34, 37), due to a large norm database and using a rigorous protocol according to the DFNS (23, 29). Thus, only small deviations from normal values can be detected for the perception performance of different mechanical and thermal stimuli, independent of sex, age, and area, respectively (17, 18, 23, 24, 28, 38). QST has been recently recommended, e.g., for the assessment of sensory small fibre neuropathies (22). As it is a non-invasive

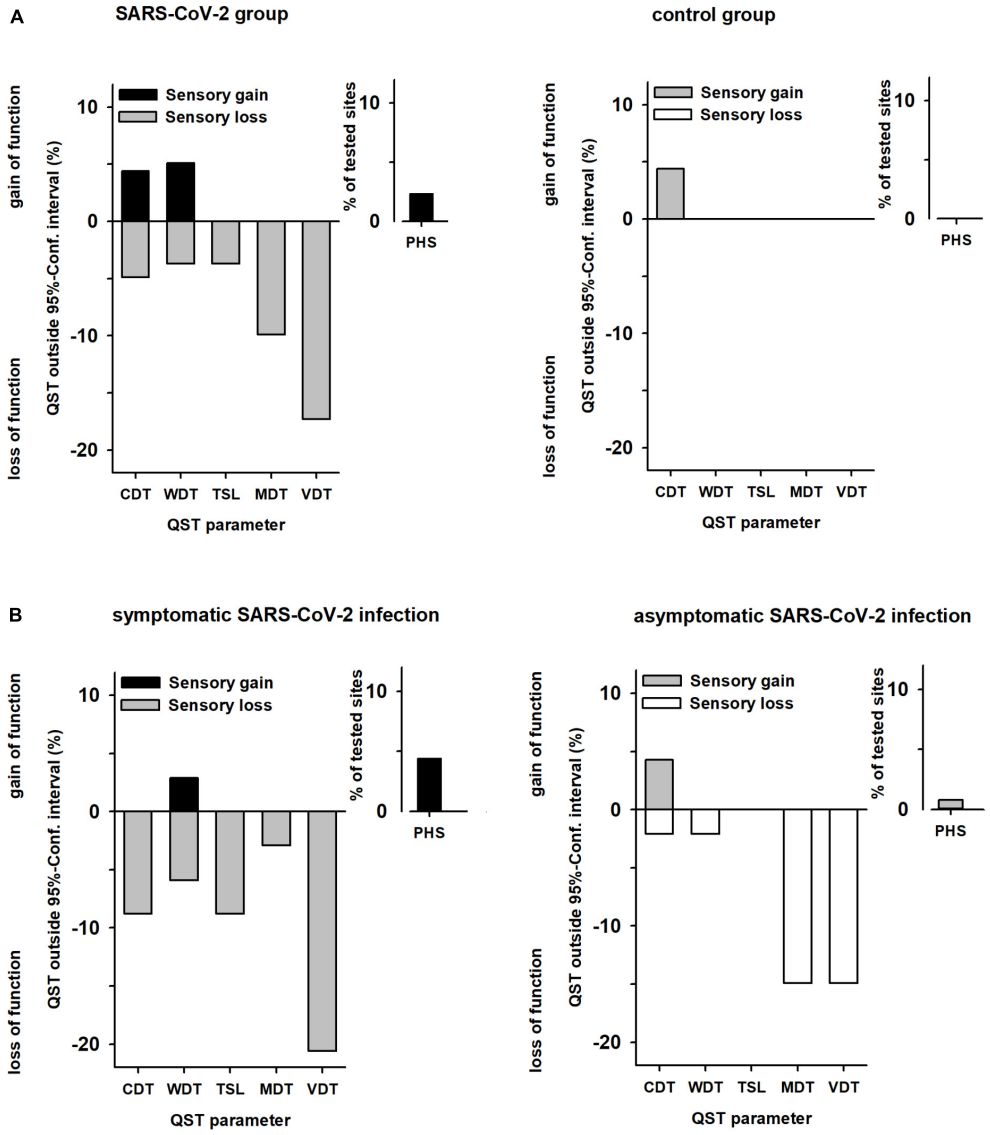

FIGURE 3
Abnormal values of sensory parameters in **(A)** patients vs. controls and **(B)** patients with symptomatic vs. patients with asymptomatic SARS-CoV-2 infection. QST values outside the 95% confidence interval (95% CI) of the reference data base (13). The *y*-axis shows percentage of subjects, with positive sensory signs mapped upward (gain of sensory function) and negative sensory signs mapped downward (loss of sensory function). **(A)** Patients *n* = 82, Controls *n* = 38. **(B)** Patients with symptomatic SARS-CoV-2 *n* = 35, Patients with asymptomatic SARS-CoV-2 *n* = 47. Absence of paradoxical heat sensations (PHS) is normal, so there are no negative signs for PHS. QST, quantitative sensory testing; CDT, cold detection threshold; WDT, warm detection threshold; TSL, thermal sensory limen; MDT, mechanical detection threshold; VDT, vibration detection threshold; PHS, paradoxical heat sensations.

and almost risk-free method, QST is a suitable tool for paediatric research, that has been used to describe the age- and sex-dependent development of somatosensory perception in healthy children (24) and evaluate somatosensory function in children and adolescents, e.g., with type I diabetes mellitus (25). In this entity, tactile hypoesthesia predicted abnormal findings in nerve conduction studies with high specificity and sensitivity, even in children without symptoms of manifest neuropathy, indicating that children have a greater ability to compensate for peripheral sensory loss of function. Our findings of abnormal somatosensory function in patients without complaints correspond to the findings in childhood diabetic neuropathy (25).

Although nerve conduction studies and skin biopsy, the latter assessing epidermal nerve fibre density in suspected small fibre neuropathy, remains the gold standard for diagnosing a neuropathy (39), these methods are not easily feasible in children without strong clinical indications. Hitherto, there

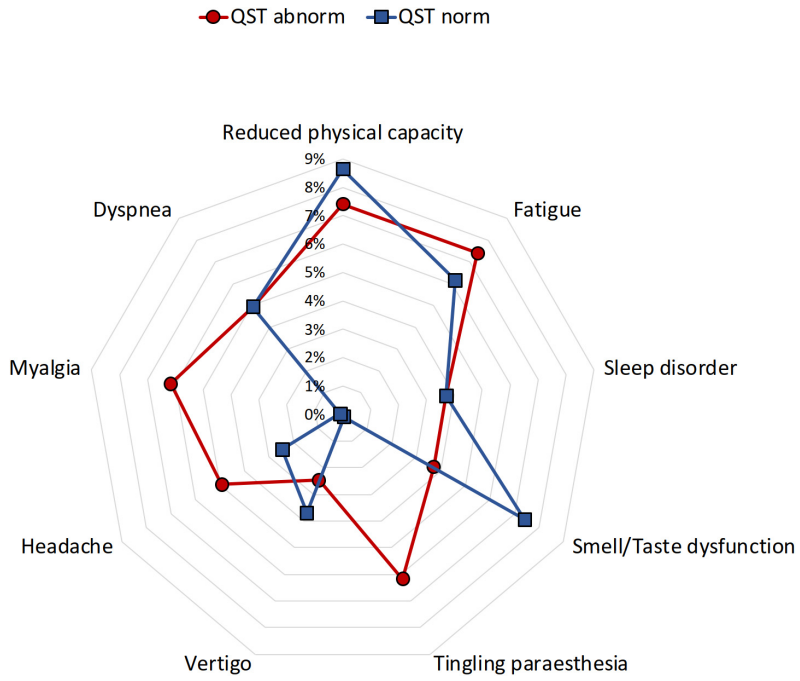

FIGURE 4
Distribution of frequent post-COVID complaints in children and adolescents with and without abnormal QST. QST, quantitative sensory testing. Normal QST (blue), abnormal QST (red).

are no data available in children with persisting complaints after SARS-CoV-2 infection. However, our results are in line with a recently published observational study in adults using corneal confocal microscopy, which reported morphological aberrations of the corneal innervation, that is, loss of small fibres and increased dendritic cells, the latter indicating immune-mediated pathomechanisms (40). In first small case series of adult long-COVID patients with new-onset paraesthesia, reduced intraepidermal nerve fibre densities were also detected in skin biopsies (41, 42).

It is undisputed, although less common in children than in adults, that SARS-CoV-2 can lead to severe neurological complications involving the central and autonomic nervous systems (43–46). Like other neurotropic viruses (Epstein-Barr virus, herpes, and influenza viruses) (47), corona virus has been reported to cause serious central nervous system disorders such as acute disseminated encephalomyelitis or myelitis in children (5, 6). An immune-mediated peripheral nerve dysfunction due to SARS-CoV-2 infection has been described only in case reports of Guillain-Barré syndrome and its variants with acute axonal neuropathy or cranial nerve affections or in children after paediatric inflammatory multisystem syndrome, where in 40% of cases, an unspecified involvement of the peripheral nervous system has been described (5, 6). Polyneuropathy syndromes have been previously described after influenza or EBV infection (9, 10). In adults, a significantly higher frequency of EBV reactivation has been recently reported in patients with long-COVID syndrome than in controls without persisting complaints after SARS-CoV-2 infection (48). Our findings suggest that somatosensory dysfunction is a common sequela of acute SARS-CoV-2 infection. It is presumably more frequent after symptomatic infections and may explain some of the most frequent reversible complaints, such as reduced physical performance, better than rare long-term pulmonary sequelae (4).

Limitations

One limitation of our study is a probable recruitment bias, as those families whose children complained of persistent symptoms after infection were more likely to visit our special outpatient clinic and report for participation. Thus, our results cannot be used to estimate the prevalence of neurological dysfunction after SARS-CoV-2 infection. Moreover, the control group was heterogeneous, and details about the previous infections were not available in some subjects, apart from SARS-CoV-2 being excluded. In contrast to two recently reported larger healthy control cohorts, none of our controls complained of reduced physical activity, fatigue, and headache as possible post-pandemic symptoms (2, 49). Furthermore, the detection thresholds of the control group were within the normal range in contrast to the SARS-CoV-2 group. Therefore, the chance of false-positive QST findings in the

group with a history of SARS-CoV-2 infection seems to be quite low. Due to the lack of additional assessment in our cohort, the origin of the impairment of sensory function cannot be specified. Further studies should include electrophysiological examination, morphological investigations of the nerve fibre density, for example by CCM, as well as profiles of antibodies against neural structures in serum and/or cerebrospinal fluid, which could possibly further elucidate the functional somatosensory abnormalities in the present study. Nevertheless, our data contribute to a better understanding of the effects of SARS-CoV-2 on the somatosensory system.

Conclusion

This study demonstrates that loss of somatosensory function for both small and large nerve fibres and the corresponding pathways are detectable approximately 3 months after mild symptomatic and asymptomatic SARS-CoV-2 infection. Somatosensory abnormalities seem to occur independently of the severity of acute infection and mostly remain unnoticed. However, our findings indicate that some persistent symptoms in children and adolescents with long-COVID syndrome may have a somatic correlate. Therefore, in long-lasting or disabling cases with particular complaints such as myalgia or paraesthesia, nerve function should be considered as part of the diagnostic work-up after SARS-CoV-2 infection. Fortunately, the somatosensory changes seem to be reversible spontaneously over time in the majority of cases.

Data availability statement

The original data presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Ruhr University Bochum (Reg. no. 20-6927_1). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LE, CM, FB, EE-K, and TL: conceptualisation. LE, CM, EE-K, and TL: methodology. LE, EE-K, and CM: formal analysis. LE, LK, and AS: investigation. LE: writing – original draft preparation. EE-K, CM, FB, AS, and TL: writing – review and editing. EE-K and TL: supervision. FB and TL: project administration. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.977827/full#supplementary-material

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Case Report: Congenital disseminated tuberculosis neonate born to tuberculosis-COVID-19 mother

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We report a case of a 26-day-old male neonate with high suggestive of congenital disseminated tuberculosis (TB) [tuberculous meningitis (TBM), pulmonary TB, and ocular TB] at term, low birth weight born cesarean section from a confirmed COVID-19 and pulmonary TB mother who hospitalized in the isolation room and never seen her son for three weeks. The baby had a fever for two weeks after birth and a history of seizures. A nasopharyngeal swab for RT-PCR SARS-CoV-2 yielded a negative result. He was initially diagnosed as having a sepsis-like syndrome and then hospitalized. Chest x-ray revealed bilateral infiltrate, cerebrospinal fluid analysis (CSF) showed clear, cell count was 9 with dominant mononuclear cell, and gastric lavages did not yield acid-fast bacilli. X-pert MTB/RIF from gastric lavage specimen detected *Mycobacterium tuberculosis* rifampicin sensitive. Anti-tuberculosis drugs for TBM were started. Abdominal sonography identified multiple hypoechoic nodules in the liver and spleen. Neuroimaging studies did not identify hydrocephalus, meningeal enhancement, infarct, or tuberculoma. A Video-EEG examination showed electrical seizure after initiation of phenobarbital. Video-EEG evaluation showed no epileptiform discharge. Upon follow-up, he showed slightly delayed motor development, pan-uveitis, retinal detachment, and cataracts. We assumed that ocular TB resulted from a paradoxical reaction following TB treatment. Retinal detachment was improved and lens replacement was done.

KEYWORDS

tuberculosis, COVID-19, meningitis, disseminated, congenital

Introduction

The COVID-19 pandemic has had a major impact on healthcare delivery worldwide. The risks to the fetus due to COVID-19 during pregnancy are not fully known, and co-infection with pulmonary tuberculosis can further complicate the situation (1). The actual incidence of congenital TB has rarely been reported (2). It has a significant mortality rate of up to 50% (3, 4). *Mycobacterium tuberculosis* can spread beyond the pulmonary into the systemic circulation, causing the dissemination of infection involving the gastrointestinal tract, spleen, kidney, adrenals, bone marrow, and

meninges (3, 5). The sepsis-like syndrome can be present if there is widespread dissemination (5). Disseminated TB is an important cause of morbidity and mortality in developing countries, especially among children under five years (5). Early identification and management are essential to prevent ongoing disease transmission (5). The diagnosis of congenital TB is challenging due to the non-specific presentation in infants, hence it is necessary to monitor pregnant women with TB during this pandemic with early detection of TB in newborns to prevent further and more severe TB (2–4, 6).

During the pandemic, TB and COVID-19 tests can be carried out simultaneously because of the same clinical features of both diseases, simultaneous exposure to both diseases, and the presence of risk factors for a poor outcome for both diseases (7).

Case presentation

A 26-day-old male neonate was brought with complaints of recurrent fever and a history of seizures since two weeks old. He was born at term, 2,400 gr, *via* cesarean section to a confirmed COVID-19 and TB mother. A nasopharyngeal swab for RT-PCR SARS CoV-2 of the baby was negative. He was discharged in good condition from the hospital on the 3rd day and was separated from his mother for 3 weeks. He was never given TB prophylaxis after birth.

At the presentation, he was alert and feverish. No abnormal respiratory findings nor seizures, but tachycardia was identified. An abdominal examination revealed liver and spleen enlargement. The laboratory findings showed decreased platelet counts, an elevated liver function test, and an increase in total bilirubin and c-reactive protein. The HIV serology result was non-reactive, while the viral load was not investigated. A chest x-ray revealed bilateral pneumonia (**Figure 1**) and an abdominal radiograph concluded a liver enlargement. He was initially diagnosed with late-onset neonatal sepsis and was given a course of empiric antibiotics. The patient had previously received phenobarbital 20 mg/kg body weight at the previous hospital and was immediately referred to our hospital. The drug was discontinued when the seizures stopped without an EEG examination. On the 3rd day of hospitalization, he had tonic posturing, suggestive of meningitis. We did a lumbar puncture and the CSF was clear with increased cell count (9 cells/HPF, mononuclear cells dominant). An X-pert MTB/RIF from gastric lavage detected rifampicin-sensitive *Mycobacterium tuberculosis*. He has been diagnosed with TBM and decided to start a TB drug regimen. However, we could not give rifampicin and isoniazid due to the increase in ALT and direct bilirubin level, so he was given levofloxacin and ethambutol. The patient did not show any sign of a seizure. However, the video-EEG result showed an electrical seizure (**Figure 2**). A brain MRI did not show any abnormalities or signs of meningitis.

After starting the TB regimen, his condition slowly improved. The fever was down and there were no clinical seizures. Abdominal sonography showed multiple hypoechoic nodules that were suggestive of liver and splenic TB. He was then diagnosed with suspected congenital disseminated TB. During hospitalization, his liver function improved. Rifampicin and isoniazid were introduced and were well tolerated. After 30 days of hospitalization, evaluation using the EEG showed no epileptiform discharge, and phenobarbital was discontinued. On day 33, he was discharged in good condition.

At three months old, he showed poor neurodevelopmental progress with gross motor delay and poor visual acuity test results (i.e., not following light). We referred the patient for a complete ophthalmology examination which revealed pan-uveitis complicated with cataracts, and exudative retinal detachment of the left eye (**Figure 3**). He was given levofloxacin, prednisolone acetate, and homatropine hydrobromide eye drops. He also underwent lens replacements for cataracts and the retinal detachment was resolved.

At six months, a follow-up MRI was normal. Brain Evoked Response Audiometry (BERA) was also normal. Amiel-Tison's neurological assessment showed mild abnormal motoric development with slight spasticity. He still takes the TB regimen regularly and we continue the therapy for 12 months.

His mother had a cough symptom since the 6th month of pregnancy but was not evaluated for TB. A cesarean section was planned because of breech presentation. Three days before the cesarean section, she had a low-grade fever, and the PCR result prior to the cesarean delivery was positive. Because the mother's chest x-ray after the delivery showed an infiltrate that was suggestive of TB, further evaluation for TB was done. The AFB sputum smear result was positive, so she was started on a TB regimen. Unfortunately, this was not accompanied by a TB investigation for the baby. The baby was discharged without TB prophylaxis. The placenta was not examined in the previous hospital. A further contact TB investigation result from her family was negative.

Discussion

Pregnant women are at increased risk of developing TB, and this is associated with poor outcomes including premature birth, intrauterine growth retardation, and low birth weight. COVID-19 and tuberculosis co-infection in pregnancy is rare (1, 8). In the general population, one study from China reveals the TB prevalence among COVID-19 patients ranged between 0.47 to 4.47% %. TB status might play a role in the development of

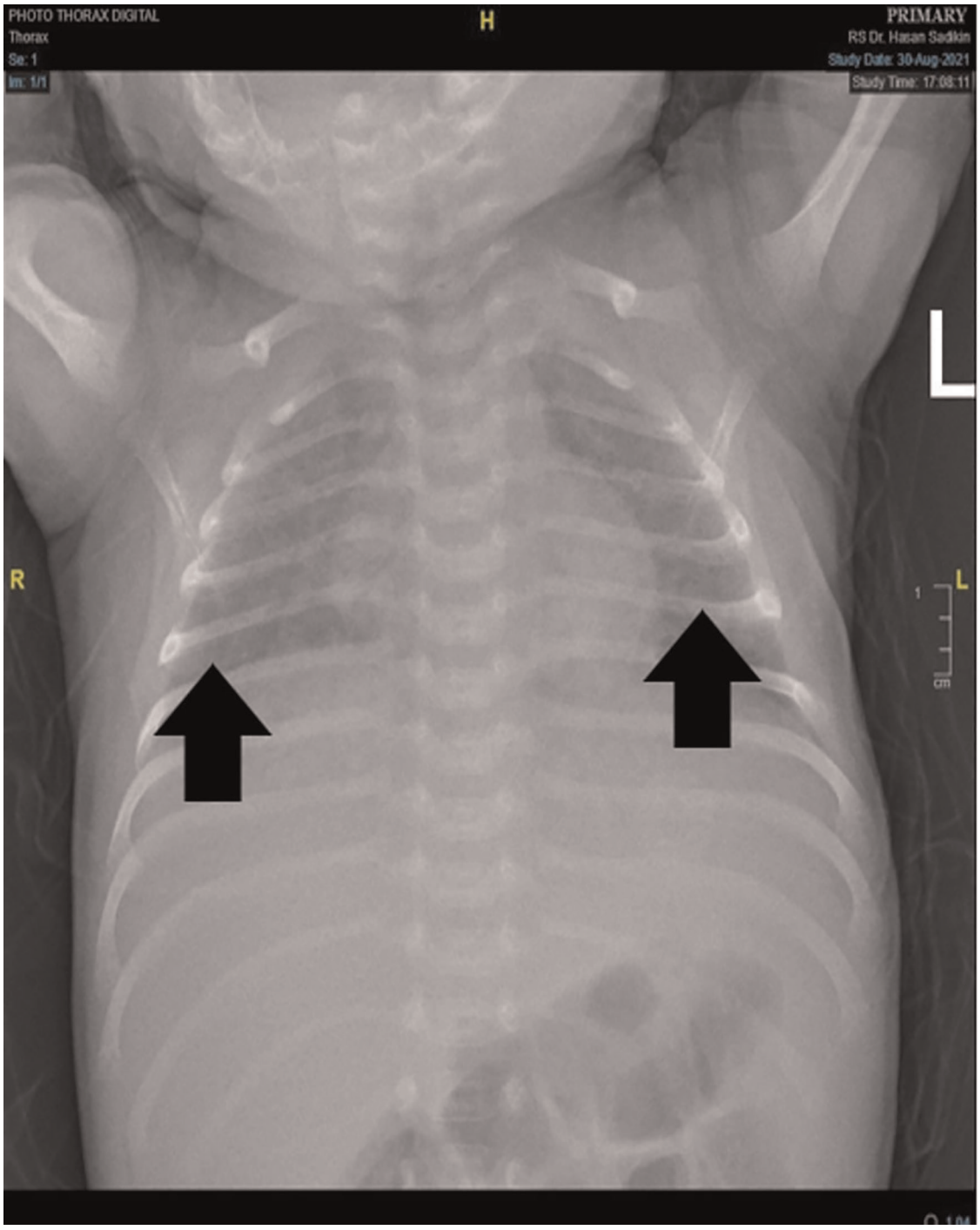

FIGURE 1
Chest x-Ray. showed inhomogeneous opaque coverings within upper and lower lung fields bilaterally (black arrow).

COVID-19 infection, exacerbation of the course of the disease, and vice-versa (9).

Here, we described the outcome of an infant with congenital disseminated TB born from a mother with COVID-19 and TB co-infection. Immune suppression caused by the SARS-CoV-2 virus may result in certain difficulties in the diagnosis and treatment of tuberculosis. Furthermore, long-term lymphopenia, hyperinflammation, lung tissue injury, and imbalance in CD4+ T cell subsets associated with COVID-19 could propagate M. tuberculosis infection and disease

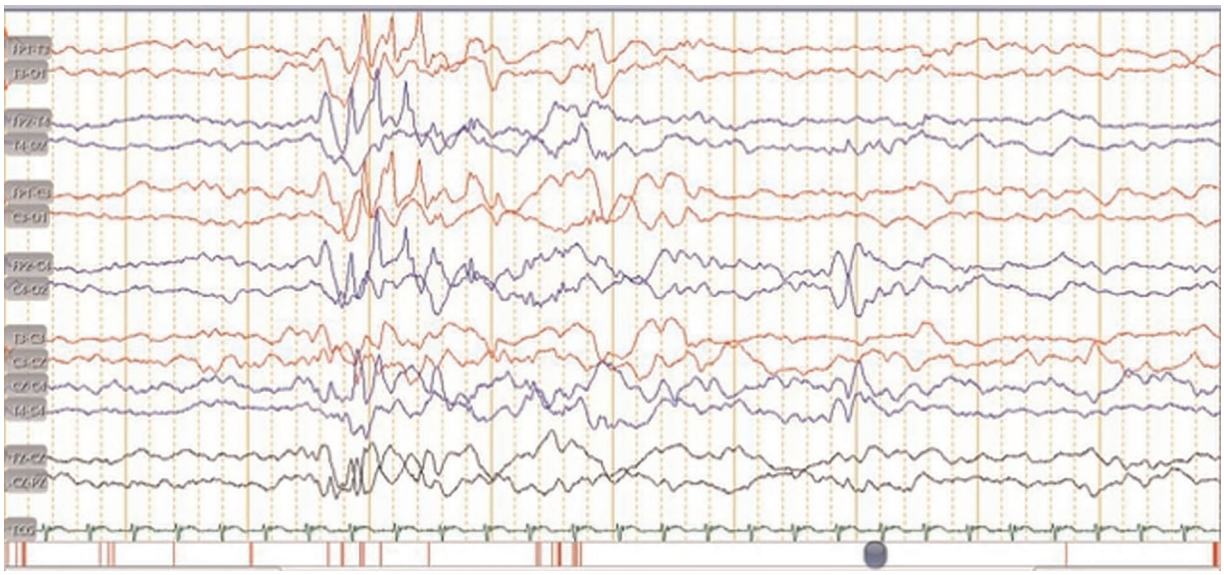

FIGURE 2
EEG in a month-old male with congenital disseminated TB showed there was an electrical seizure.

progression (10). The nasopharyngeal swab for RT-PCR SARS CoV-2 of the baby was negative, which likely indicated that no vertical transmission occurred (11). Congenital TB is a fatal disease that must be diagnosed as early as possible to avoid devastating outcomes (2–4). In a newborn child, the clinical presentation of the disease is non-specific, which often delays the diagnosis (6, 12). The transmission is most commonly from hematogenous spread *via* the umbilical vein, infected amniotic fluid aspiration, or ingestion of infected secretions (6, 12). The primary complex in the liver, along with caseating granuloma, is the definitive lesion of congenital tuberculosis (2–4). Unfortunately, we cannot prove this case was congenital in origin as we did not perform the liver biopsy and placental histopathology examination to find caseating tubercles for diagnosing congenital TB. The suspicion of TB in pregnant mothers is necessary for diagnosing TB in neonates and must be considered in countries where TB incidence is high (13). The most common symptoms attributed to congenital TB are fever (70%), hepatic and/or splenic enlargement (67%), and lethargy with periods of irritability (40%), as identified in our case (3, 5).

Cantwell modified the criteria diagnosis of congenital TB; it requires (a) *Mycobacterium tuberculosis* lesion and (b) 1 of the following secondary findings: (1) Primary hepatic complex (caseating granuloma) on biopsy, (2) lesions from any source (i.e., pulmonary, hepatic, and skin) in the first weeks after birth, (3) exclusion of postnatal transmission through a thorough investigation of contacts, or (4) TB infection of the maternal genital tract and/or placenta (3, 14). Congenital TB, in this case, has not been established because the histopathological examination of the placenta was not performed. However, we excluded post-natal transmission by thorough investigation through history taking and AFB sputum smear. In this case, we assumed he had TB congenitally transmitted, and the patient had developed symptoms of TB since the age of 2 weeks before contact with his mother. The age at the beginning of congenital TB is not uniform. Infants with congenital TB may be asymptomatic at birth, but symptoms can occur within days to weeks after birth. This is due to the different immune statuses of each newborn and the onset of disease may be slower in some children (15). The symptoms of congenital TB mainly occur until 3 months after birth, at an average age of 28 days with the longest duration between birth and the onset of symptoms being 154 days (15). He had severe manifestations of congenital disseminated tuberculosis (i.e., TBM, pulmonary TB, and ocular TB).

Active TB in pregnancy is associated with adverse maternal and fetal outcomes. Early diagnosis of TB is important to prevent significant maternal and perinatal complications. Newborns from mothers with pulmonary TB must be given tuberculosis preventive treatment (TPT) and should be separated until both have been evaluated (14, 16).. Unfortunately, the patient did not receive TPT from the previous hospital.

Congenital TB could develop into meningitis TB with seizures as its primary symptoms; therefore we should consider the possibility of meningitis TB (12, 14). Neonatal seizures, if prolonged and untreated, can cause permanent damage due to decreased oxygen flow and excessive brain cell

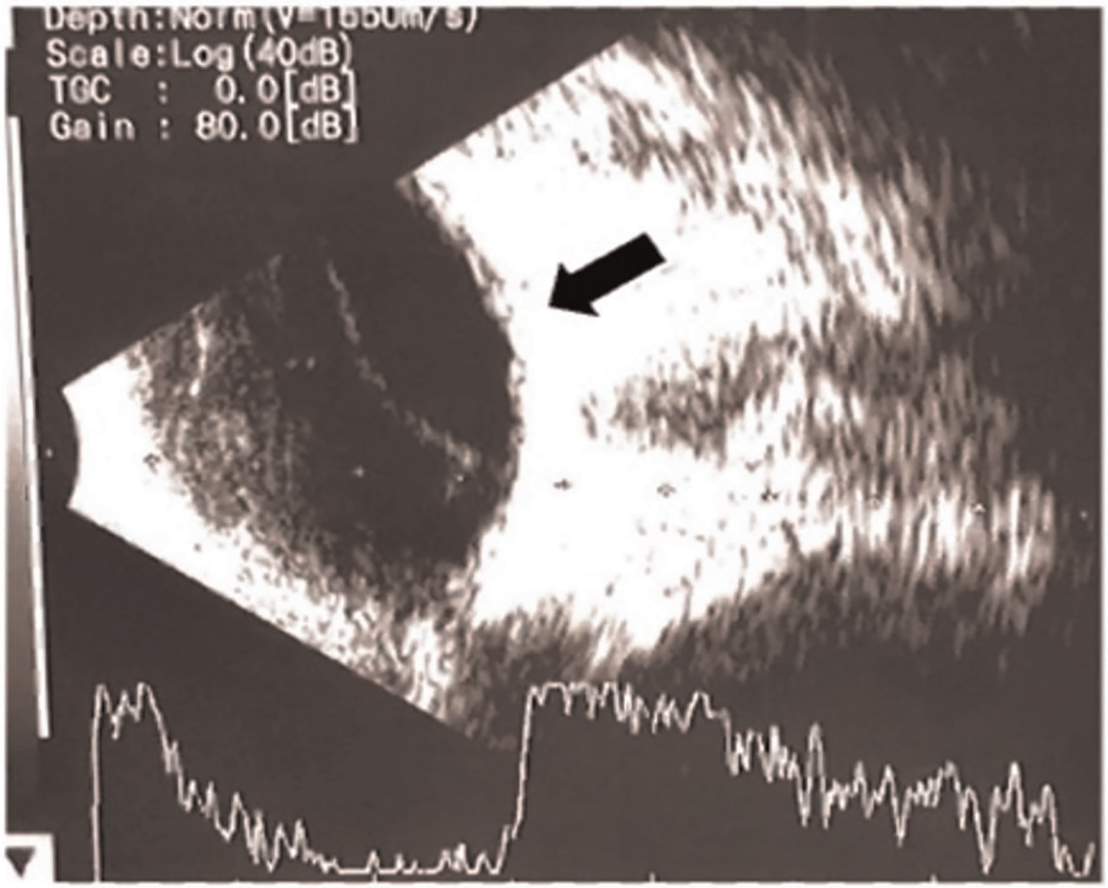

FIGURE 3
Ultrasound examination of the left eye showed pan-uveitis developed exudative retinal detachment.

activity. Subtle or electrical seizures are common in neonates, described as subtle because the symptoms are frequently overlooked (17). Electrographic or electrical seizures, namely EEG electrical seizure activity without apparent clinical manifestation, are more common after the initiation of an antiepileptic drug such as phenobarbital (17).

The anti-epileptic drug can suppress the clinical manifestation of seizure but not the EEG ictal discharge (17). An electrographic seizure could be a sign of poor prognosis (17). We suspected that ocular TB in this patient was due to a paradoxical reaction following TB treatment. In the beginning, the patient's eyes were found to be normal but during monitoring, there were complications in the patient's eyes (i.e., pan-uveitis, cataracts, and retinal detachment). A paradoxical reaction in patients with TBM is characterized by the worsening of pre-existing tuberculous lesions or the appearance of new tuberculous lesions in patients whose clinical symptoms initially improved and had been on anti-tuberculosis treatment for at least 10 days (18). This case illustrates the important obstacles in managing a newborn with TB. A placenta examination was not performed, resulting in the delay of diagnosis, and not giving TPT may worsen the patient's outcome. A bone marrow puncture (BMP) was not performed due to decreasing platelet count of <50.000/mm^3. This case showed up on monitoring, and various complications were discovered. Therefore, this is an important issue that must be considered to improve the diagnosis and treatment that can reduce morbidity and mortality in TBM.

Our limitation is that we do not know whether TB in this patient is of congenital origin or not because there is no confirmation data from placental histopathology.

Conclusions

In this case, we described a baby from a COVID-19 and TB-positive mother with severe congenital TB. It is necessary to monitor pregnant women with TB during this pandemic and early detection of TB in newborns to prevent further and more severe TB. It is important to follow up with TB patients

to ensure that a paradoxical reaction does not occur as it did with this patient. Despite the severity of congenital TB, prompt and rigorous treatment of seizures and the management of eye complications had good outcomes in this case.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical review and approval were not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the parents.

Author contributions

HMN: conceptualized and designed the study and was involved in first drafting the manuscript, and critical revision; NAR, RDF: was involved in drafting the manuscript and critical revision All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Seroprevalence of SARS CoV-2 among children after the second surge (June 2021) in a rural district of South India: Findings and lessons from a population-based survey

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Objective: To determine the seroprevalence of SARS COV 2 among children in the Bangalore Rural district.

Methods: We conducted a cross-sectional study after the second surge of COVID-19 from 14 June to 13 July 2021 and recruited 412 children through house to house visits from four villages in a rural district. We administered a questionnaire to collect demographics and details of COVID-19 infection and used the ABCHEK Antibody Card test (NuLifecare,India) which is an ICMR approved test for detecting antibodies (IgG & IgM) by immunochromatography using the finger prick method. We used Statistical Package for the Social Sciences version 20.0 for analysis.

Results: Our participants had an age group ranging from 11 months to 18 years. There was an almost equal distribution of boys (48.3%) and girls (51.7%). We estimated seroprevalence of 45.9% (95% CI: 41–50.8) among children. Seroprevalence was significantly associated with a history of symptoms suggestive of COVID-19 in the past, the seropositive status of the parents, and any other family members being tested positive. Age and gender of the child, education and occupation of the parents were not associated with the seropositivity status of the child.

Conclusion: Seroprevalence of COVID-19 among children corresponds to adult seroprevalence during the same time interval. This knowledge can be of practical application where adult prevalence is documented. Unvaccinated children in low-resource settings need special attention with respect to monitoring for new mutations as well as managing endemic needs.

KEYWORDS

COVID-19, COVID-19 in children, seroprevalance, rural India, pandemic (COVID19)

Introduction

In India, the first case of COVID-19 was reported on 30 January 2021. As of 09 September 2022, India had documented 44 million COVID-19 cases and 0.5 million COVID-19 deaths (1). By that time, India had seen two waves, first in late 2020 and then in early April of 2021. The second pandemic wave was far more devastating because of accelerating cases and a crunch for life-saving hospital beds and supplies (2, 3). Infection in the younger population, happy hypoxia and a relatively sudden reduction in oxygen saturation, increased opportunistic infections like mucormycosis and a higher number of deaths made the second wave direr than the first wave (4, 5).

Since the second wave was graver than what the experts and the public expected, the thought of a third wave still lingered on everyone's minds even though the ICMR 4th serosurvey reported a seroprevalence of 67.6% (6). Documented high seroprevalence and vaccination among adults reduced the possibility of a third wave of high amplitude. In this context, one of the important considerations was the vulnerability of children with COVID-19 as they formed a significant proportion of the unvaccinated population. There was limited seroprevalence data among children at the time of conducting the study. Since then, outbreaks of COVID-19 have been identified in different countries in schools and day care centres. There are reports which indicate that younger children may be less infectious, as measured by secondary attack rates, than adolescents and adults (7).

Though SARS-CoV-2 is thought to cause less severe illness and fewer deaths in children and adolescents compared to adults, it's important to note the multisystem inflammatory syndrome in children (MIS-C) reported in many countries, a severe manifestation of the virus. Although it affects multiple systems, the cardiovascular signs are the most prominent. These patients exhibit high anti-SARS-CoV-2 antibody titres an it mimics Kawasaki disease (KD). Being a novel disease entity, the immunopathogenesis of this condition is not fully understood (8). In addition to being critically ill requiring ICU admissions, the mortality has been as high as 9% in children in with MIS-C (9).

Children and adolescents also remain susceptible to the infection and form a part of the transmission chain. The risk of both infection and transmission is reported to increase with age. As reported to the WHO, during the initial pandemic phase with the ancestral strain during the time period from 30 December 2019 to 25 October 2021, children under five years of age represented 2% of reported global cases. Older children (5 to 14 years) accounted for 7% and older adolescents and young adults (15 to 24 years) represented 15% of reported global cases (10, 11). Persistence of anti–SARS-CoV-2 spike receptor-binding domain IgG was seen in a household cohort study in Italy even following asymptomatic infections until 12 months after infection in all age groups and higher levels of binding antibodies were seen in children younger than 3 years when compared with adults older than 18 years. In addition to direct benefits, vaccinating children would be expected to decrease transmission in this age group and also transmission from children and adolescents to older adults. COVID-19 (12).

The Community Health Division (CHD) of Bangalore Baptist Hospital has been providing curative and preventive health services through a Rural Health Centre and network of mobile clinics to residents of Bangalore rural district for over a decade. Though these rural areas were spared in the first wave (seroprevalence 12%), the virus swept through the rural landscapes, affecting most of the population during the second surge (13). A serosurvey in June 2021 reported a prevalence of 63.2% among adults in Bangalore rural district (14). When the report was communicated to the community in the surveyed area, the community was interested in knowing whether their children were infected during the delta surge. Hence we designed a study with the objective to determine the seroprevalence of COVID-19 among children in the Bangalore Rural district. The result of the study is of interest to people in the communities and local authorities for resource planning purposes. Our findings will be of research and policy importance as there are limited publications regarding COVID-19 transmission and seroprevalence among children from rural India.

Methodology

We conducted a cross-sectional study based on WHO's recommendations in the Bangalore rural district. A detailed description of this district, including population, occupation and health care services provided, can be found in our earlier publication (13). We randomly selected one of the four sub-districts and then randomly chose four villages from the selected sub-district. Based on our earlier adult survey, we assumed a seroprevalence of 30%, with a relative precision of 10% and a design effect of 1.2; the sample size was calculated as 403. The study was approved by the Ethics committee of Bangalore Baptist Hospital on 23 June 2021.

We obtained parental consent for children under 14 years and parental consent and verbal assent from the participants between 14 and 18 years. We recruited all the eligible children (0–18 years) through a house-to-house survey from 14 June to 13 July 2021 after the required consent/assent. Children who were seriously ill with any other chronic conditions or had any established comorbidities were excluded. We administered a questionnaire on demographics (age, gender, education of the parents) and the history of COVID-19 infection among the participants and family members. The

TABLE 1 Age-wise distribution of unadjusted seroprevalence.

	Category	Male	Prevalence (95% CI)	Female	Prevalence (95% CI)	Total	Overall prevalence (95% CI)
Age (years)	0–5	44	54.5 (38.8–69.6)	39	61.5 (44.6–76.6)	83	57.8 (46.5–68.6)
	6–10	80	35.0 (24.7–46.5)	65	36.9 (25.3–49.8)	145	35.9 (28.1–44.2)
	11–15	65	43.1 (30.8–56.0)	64	57.8 (44.8–70)	129	50.4 (41.5–59.3)
	16–18	24	54.2 (32.8–74.4)	31	35.5 (19.2–54.6)	55	43.6 (30.3–57.7)
	Total	213	43.7 (36.9–50.6)	199	48.2 (41.1–55.4)	412	45.9 (41.0–50.8)

interviewer used Epi-info 7.0 TM mobile application-based tool to record replies offline, which were later downloaded for analysis. We used an ICMR approved point of care card test, ABCHEK Antibody Card [NuLifecare, Noida (UP), India], for detecting IgG and IgM antibodies by immunochromatography using the finger prick method (15). We counselled the parents and children and reassured them in order to reduce the anxiety and pain due to needle prick before collecting the sample. The test has a total co-incident rate of 92.8% and 96.5% for IgM and IgG antibodies, respectively, as per the manufacturer (15).

This test kit was evaluated in our laboratory using the Elecsys SARS CoV-2-S assay which tests for total antibodies including IgM and IgG (Roche Diagnostics). The measurement range of the assay is from 0.40 U/ml to 250 U/ml. Levels of <0.80 and ≥0.80 U/ml were considered as negative and positive respectively according to the manufacturer's recommendations (16). The evaluation was done with a total of 30 samples of known serostatus. All the seronegative samples and samples with total antibody levels greater than 160 U/ml gave concordant results. The card test could not detect antibody levels less than 160 U/ml.

We used Statistical Package for the Social Sciences version 20.0 for analysis. The unadjusted COVID-19 IgG antibody's seroprevalence was reported in percentage with a 95% confidence interval (CI). Using chi-square tests, the relationship between seroprevalence and comorbid conditions and socio-demographic characteristics were investigated.

TABLE 2 Factors associated with seropositivity among children.

Factors	Categories	Serological status		Total	*p*-value
		Reactive	Non - Reactive		
Age in years	≤10	100 (43.1)	128 (56.1)	228	0.35
	>10	89 (48.4)	95 (51.6)	184	
Gender	Male	93 (43.7)	120 (56.3)	213	0.36
	Female	96 (48.2)	103 (51.8)	199	
Education of the father	Lower (≤10 years)	132 (44.6)	164 (55.4)	296	0.40
	Higher (>10 years)	57 (49.1)	59 (50.9)	116	
Education of the mother	Lower (≤10 years)	121 (46.9)	137 (53.1)	258	0.58
	Higher (>10 years)	68 (44.2)	86 (55.8)	154	
Occupation of the father	Farmer	60 (40.8)	87 (59.2)	147	0.85
	Others	129 (48.7)	136 (51.3)	265	
Occupation of the mother	Housewife	123 (45.6)	147 (54.4)	270	0.12
	Others	66 (46.5)	76 (53.5)	142	
Father's serostatus	Reactive	32 (58.2)	23 (41.8)	55	0.04*
	NR/Not done	157 (44)	200 (56)	357	
Mother' Sero status	Reactive	42 (59.2)	29 (40.8)	71	0.01*
	NR/Not done	147 (43.1)	194 (56.9)	341	
History suggestive of COVID–19	Yes	39 (56.5)	30 (43.5)	69	0.05*
	No	150 (43.7)	193 (56.3)	343	
Tested positive	Yes	8 (66.7)	4 (33.3)	12	0.14
	No	181 (45.2)	219 (54.8)	400	
Family members tested positive	Yes	44 (60.3)	29 (39.7)	73	0.006*
	No	145 (42.8)	194 (57.2)	339	

*significant *p*-value.

Results

We conducted a serosurvey among 412 children with age groups ranging from eleven months to eighteen years. One-third were in the age group of 6–10 years (35.2%). There was an almost equal distribution of boys (48.3%) and girls (51.7%). The majority of the parents were educated till high school (fathers – 53.6%, mothers - 44.7%). More than two-thirds (65.5%) of the mothers were homemakers, and one-third of the fathers (35.7%) were farmers.

Twelve children (2.9%) reported having positive IgG status in the past, while 15% gave a history of at least one symptom suggestive of COVID-19 in the last month. Among the parents, 13.3% of fathers and 17.2% of mothers were detected as positive for COVID-19. Our Study population did not have children who lost their parent/s due to COVID-19. Similarly, 17.7% reported at least one family member was found to be positive in the past. We estimated a seroprevalence of 45.9% (95% CI: 41–50.8) among children in the rural district. We also found that the seroprevalence was higher in the age group of 0–5 years (Table 1).

Seroprevalence was significantly associated with history of symptoms suggestive of COVID-19 in the past, the seropositive status of the parents, and any other family members being tested positive. Age and gender of the child, education and occupation of the parents were not associated with the seropositivity status of the child (Table 2).

Discussion

Our study revealed a high seroprevalence (45.8%) of COVID-19 infection among children in the Bangalore Rural district. This was lower than the seroprevalence among vaccine-naive adults in the same population during the same period (63.2%) (14). The only other study conducted during the same period among children from India quoted a 55.7% seropositivity rate (using IgG ELISA) among children and a corresponding 63.5% seropositivity in adults (17). Geographic variation (different states), the difference in assays and the time period would have resulted in a slight difference in prevalence.

Many studies on the paediatric population using different methods for serologic testing, using different antigens for SARS CoV2, (nucleocapsid and spike protein), done at hospitals, schools and communities have been published. Some of these show a lower seroprevalence from 0.23% to 14.4%, including 0.23% in Australia (November 2020 to March 2021), 10.8% in Germany (October to March 2021), 8.4% in Canada (March to April 2021) and 14.4% Belgium (September to October 2020) (18–21). Some of the studies have shown a higher seroprevalence such as 37% from Melbourne, Australia (May to October 2020), where the proportion in children was lower than that seen in adults (37% vs. 72%), 46.7% in Romania (March to June 2021), where it was seen to be similar to that in adults (45.6%) and increasing from 52.8% in January 2021 to 81.8% in September to October 2021 in Delhi, India (22–24). Since the vaccination among children was started later than adults, these studies were done prior to vaccination, however a direct comparison is not possible as they have been done around the world at different time points and relating differently to the waves of the pandemic in each country or continent except to understand that there are wide differences noted in seroprevalence, and some have shown a difference when compared to adults, while others have shown similar levels of seroprevalence.

The card test that we used did not pick up antibody titres less than 160 U/ml, which would have underestimated the seroprevalence in our study. There was limited data at the time of the study regarding the level of antibody production in children when infected (25). Distinct antibody responses in children when compared to adults might also lead to underestimation of the seroprevalence (25).

The vulnerability of children in getting an infection was a debate among many experts and communities, equally. The debates on transmission and its implications are legitimate, as the paediatric segment forms the bulk of the unvaccinated population. The absolute numbers of Covid infected children documented in the second wave were higher than in the first wave; however, the proportion of children remained more or less the same during the first and second waves (26, 27). During the second wave (15 March – 31 May 2021), 8.57% of infections were reported in children in the 11–20 year age group and 3.05% in the 1–10 year group (28). In the first wave, similar proportions of 8.03% and 3.28% were reported, respectively (28). The Lancet COVID-19 Commission India Task Force said that less than 5 per cent of children would require hospital admission in COVID-19, 5% with severe disease and out of them, the mortality is 2 per cent (29). With a high seroprevalence rate as close to adults, it is likely that the virus transmits to the paediatric population effectively however there were not many symptomatic cases requiring hospitalisation among children.

Five months post-study, in the month of January 2022 (30), an omicron wave hit India, including Bangalore rural district. A handful of new cases in early December was replaced by 100,000 new cases in early January 2022 (30). Though there was a rapid explosion of cases in a few weeks' time, the hospitalisation caused by the third wave was meagre. The disease was milder in nature and in most cases, COVID-19 was an incidental cause than a reason for hospital admission, both among adults and children (31). Though the trajectory was steeper, the peak was smaller in magnitude than the second wave (347,000 as compared to 414,000 in the second wave) (32). India's third wave trajectory is remarkably different from most other badly affected countries, where the peak of the Omicron wave was two to four times higher than their previous peaks. One of the logical explanations is hybrid immunity (33) - population's (both adults and children) high exposure to the virus in the delta surge and a reasonable level of vaccination (adults) prior to the Omicron wave in January 2022 (31).

Though most children suffered from milder diseases, the health system's capability to handle serious paediatric cases is limited in most Indian settings, leave alone rural areas. Rare but severe COVID-19 cases and Multi-system Inflammatory Syndrome (MIS-C post-COVID infection) will be challenging to handle in the background of an 82% shortage of paediatricians in primary health centres (34, 35). This vulnerability demands a wake-up call to build up primary paediatric healthcare capacities in rural areas. Though the virus is causing lesser consequences with time, we must not forget the vulnerability of low-resource settings. This is evident by the fact that both new mutations, Delta and Omicron emerged in less wealthy countries (36). Unvaccinated population in low-resource setting needs special attention with respect to monitoring for new mutations as well as managing endemic needs.

Health system vulnerability forced the government to shut the schools for almost one and a half years, creating a privilege gap. The price of the good intention of saving lives is a widened learning gap and an aggravated socio-economic divide (37, 38). Children from poor households have forgotten to read and write, many are redeployed to work, and others are married because of the stark digital divide (39, 40). This effect fell

disproportionately on girls and children belonging to lower castes. It is important to recognise the learning loss and put in structures to bridge the learning gap. Another important strategy is to come up with practical plans for continuing education keeping in mind the future local spike and sporadic outbreaks. Freezing to immobility by closing corridors of learning may not be a wise response to endemic COVID-19 infections.

Vaccination among children is also an essential topic of discussion as vaccine trials are progressing among children worldwide. In India, an expert panel of the Drug Control General of India has recommended granting emergency use authorization (EUA) to Bharat Biotech's Covaxin for children aged two to eighteen years with certain conditions (41). In India, COVID-19 vaccines are available for children over the age of 12 years since January 2022. As on 09 September 2022, 80 million children between 12 and 18 years of age have been administered two doses of vaccine (1). Vaccinating children has not resulted in any major adverse reactions in untoward incidents in India thus far. The advantages of vaccinating children has several indirect benefits apart from direct health benefits. It has given them access to education in physical class rooms, enhanced overall well being and reduced the anxiety of the parents. It has also helped reduced the transmission from children to adults.

The study has many strengths. Firstly, this is one of the very few COVID-19 seroprevalence studies among children from rural India, a population often underrepresented in research. Secondly, the study has a good sampling strategy (multi-stage random sampling and household visits) and an adequate sample size. Thirdly, having estimated the adult seroprevalence in the same district simultaneously sheds light on the transmissibility of COVID-19 in the paediatric population in relation to adult infection (14). The relationship between adult and child seroprevalence is valuable in making assumptions seroprevalence of one group is known. Though it is a valuable study, the study has a few limitations. The antibody card test would have underestimated the seroprevalence. However, we do not know how much it would have underestimated the true seroprevalence. Another limitation is that the findings of the study are to be extrapolated keeping in mind that the external validity is limited as it is conducted in a single rural district of Karnataka.

Conclusion

During the second surge, the study in a rural district of South India showed a high seroprevalence of COVID-19 infection among children (aged less than 18 years). It concludes that the seroprevalence of COVID-19 among children corresponds to adult seroprevalence during the same time interval. This knowledge can be of practical application where adult prevalence is documented. Though mild, the rural primary health setting should be oriented to diagnose and manage children with rare but severe Covid 19 cases and MIS-C. Considering the equity issues, vaccination among children in low-resource settings is paramount as it is the primary defence against severe disease and future mutations. Further investigation is needed to evaluate the effectiveness of vaccines and to assess the immunological response in children. The study also emphasises the need for local plans to ensure continued education and social interaction in children as future local spikes are inevitable.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Bangalore Baptist Hospital (23 June 2021). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CEG, LRI, SR, and SC conceived the study. CEG received the funding, was involved in data acquisition, analysis and interpretation, and prepared the preliminary draft of the manuscript. LRI developed the study tool, supervised the data collection, involved in data analysis and writing of the results. SR, RFJ, and SM coordinated data collection, were involved in data interpretation and edited the manuscript. SC supervised and validated the blood analysis. All authors revised the work for important intellectual content and agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Clinical characteristics and etiology of children with bronchiolitis before and during the COVID-19 pandemic in Suzhou, China

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Objective: We sought to compare the clinical characteristics and etiology of children with bronchiolitis in Suzhou before the pandemic of coronavirus disease 2019 (COVID-19) with those during the pandemic.

Methods: Children who were hospitalized with bronchiolitis in the Department of Respiratory Disease, Children's Hospital of Soochow University were retrospectively enrolled over 3 consecutive years (2019, 2020, and 2021) from February 1 to January 31. Medical records were reviewed for etiology, clinical manifestations, and laboratory examination results.

Results: The pathogen detection rate and the positive respiratory syncytial virus (RSV) detection rate were lowest in 2020 and highest in 2021. The rate of human rhinovirus detection in 2021 was higher than that in 2019 but similar to that in 2020. The RSV-positive rate differences among the 3 years varied by age group. Regarding the monthly distribution of RSV-positive cases over the 3-year study, all age groups showed a significant increase in the number of cases during the winter of 2021, and this increase started as early as October. With regard to clinical manifestations, the proportion of children presenting with stuffy nose rhinorrhea in 2021 [73.33% (165/225)] was greater than that in 2019 [48.61% (122/251)] and 2020 [57.06% (97/170)], while the proportion of children with gastrointestinal symptoms in 2021 [11.56% (26/225)] was smaller than that in 2019 [25.50% (64/251)] but similar to that in 2020 [17.06% (29/170)].

Conclusions: After the implementation of COVID-19 pandemic-related interventions, significantly lower pathogen detection and RSV-positive rates were observed in children with bronchiolitis in 2020. An upward trend in these rates was observed in 2021, coinciding with the relaxation of COVID-19 prevention measures. Strengthening infection control and surveillance systems is extremely important for future work.

KEYWORDS

bronchiolitis, clinical characteristics, etiology, COVID-19 pandemic, respiratory syncytial virus

Introduction

Bronchiolitis, an acute infection of the lower respiratory tract involving mainly the bronchioles, is one of the most substantial disease burdens for infants and young children worldwide (1). It is a common clinical syndrome occurs in children under 2 years of age, most often in the first year of life. A typical disease course begins with rhinitis and cough, which may progress to tachypnea, wheezing, rales, use of accessory muscles, grunting, or nasal flaring (2). Viral infection is the main cause of bronchiolitis; the most common causative viruses are respiratory syncytial virus (RSV), followed by human rhinovirus (HRV), parainfluenza virus (PIV, especially PIV III), adenovirus (ADV), influenza virus, human metapneumovirus (hMPV), and human bocavirus (HBoV) (3). Additionally, *Mycoplasma pneumoniae* (MP), *Chlamydia pneumoniae* (CP) and *Bordetella pertussis* (B. pertussis) can also cause bronchiolitis (4–7).

At the end of 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in hospitalized patients in Wuhan, China, and the illness caused by this virus was later named coronavirus disease 2019 (COVID-19) (8). In late January 2020, Suzhou municipal government implemented the first-level public health emergency response and ordered a stringent lockdown. Measures, such as prohibiting people from gathering, closing public places, postponing the resumption of work and school, wearing masks, and maintaining physical distancing, were taken to prevent the rapid spread of SARS-CoV-2. These measures impacted not only COVID-19, but also the dynamics of various other infectious diseases, including bronchiolitis (9, 10). However, it is unclear whether, in the second year of the pandemic when the prevention measures were relaxed, the pathogens associated with bronchiolitis continued to be markedly reduced and whether the clinical manifestations changed.

Because Suzhou municipal government enacted lockdown measures at the end of January 2020, here, we took January 31, 2020 as the time node and compared the clinical characteristics and etiology of children hospitalized with bronchiolitis over 3 consecutive years, i.e., 2019 (pre-pandemic), 2020 (first year of the pandemic), and 2021 (second year of the pandemic), from February 1 to January 31.

Research subjects and methods

Subjects

We conducted a retrospective analysis of the data from children who were admitted to the Department of Respiratory Disease at the Children's Hospital of Soochow University with a final diagnosis of bronchiolitis during the period from February 1 to January 31 in 2019, 2020, and 2021. It is important to note that only patients with negative SARS-CoV-2 nucleic acid tests could be admitted to the hospital. The inclusion criteria for this study were: age of between 1 month and 1 year old; occurrence of first episode of wheezing; and clinical evidence of bronchiolitis (tachypnea, wheeze, prolonged expiratory phase, and crackles on auscultation). The exclusion criteria were: recurrent wheezing; bronchopulmonary dysplasia; neuromuscular disease; congenital disease; congenital immunodeficiency; or evidence suggesting that the wheezing is caused by non-infectious factors, such as a bronchial foreign body. This study was approved by the Medical Ethics Committee of the Children's Hospital of Soochow University.

Specimen collection

Nasopharyngeal secretions, collected from all children within 24 h of hospital admission, were analyzed. For aspiration, a suction catheter was introduced through the nose and advanced into the lower portion of the pharynx, up to a distance of approximately 7 cm–9 cm, and approximately 2 ml of nasopharyngeal secretions were obtained and sent for analysis within 30 min. The retrieved specimen was centrifuged (500 g, 10 min) and suspended in 2 ml saline before being divided into two aliquots for pathogen identification using a direct immunofluorescence assay (DFA) and polymerase chain reaction (PCR).

Microbe detection

Seven common respiratory viruses, i.e., RSV, influenza virus A, influenza virus B, PIV I, PIV II, PIV III, and ADV were all detected using DFA. Chemicon (United States) provided the assay kits, and all staining methods were carried out according to the manufacturer's instructions. After that, immunofluorescence experiments were carried out (Leica 020-518.500, Germany). Trizol reagent (Invitrogen, United States) was used to extract RNA from the specimens, which was followed by reverse transcription to create cDNA. The cyclic temperature settings were 94 °C for 30 s, 55 °C for 30 s, 68 °C for 30 s, and a final extension at 68 °C for 7 min after 45 amplification cycles. A fluorescent real-time PCR (BIO-RAD iCycler) was used to detect hMPV and HRV. To identify HBoV, DNA extraction and real-time PCR were used. The 16 s rRNA gene of MP extracted from nasopharyngeal samples was identified using a quantitative diagnostic kit (Daan Gene Co., Ltd. Of Sun Yat-Sen University) for MP DNA.

Data collection

The medical records of the patients were reviewed, and data regarding the following parameters were recorded: (1) demographic and clinical characteristics, including age, gender, symptom duration prior to admission, and hospital stay length; (2) results of blood tests for inflammatory profiles, including white blood cell (WBC) count, neutrophil percentage, and serum C-reactive protein (CRP) level; and (3) results of etiological examination of nasopharyngeal secretions. Tachypnea was defined as follows: >60 breaths/min in children aged <2 months, >50 breaths/min in children aged 2–11 months.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Science (version 26.0, IBM Corporation, Armonk, NY, United States). Descriptive continuous outcome variables are presented here as medians (IQR). If the measurement data met the requirements of normality and homogeneity of variance, two independent sample T tests were performed; if not, a Kruskal-Wallis H test was performed. A chi-squared (χ^2) test or Fisher's exact test was used to analyze categorical data. *Post-hoc* multiple comparisons were performed to determine the origins of significant differences, and the results were adjusted by using the Bonferroni method. *P*-values of <0.05 were taken to indicate statistical significance. Graphs were produced with GraphPad Prism (Version 8.4.3, GraphPad Software Inc).

Results

Demographic characteristics

Among the 2,272 children under 1 year of age who were hospitalized during the 3-year study period, 787 (34.64%) were diagnosed with bronchiolitis: 300 (38.12%) in 2019, 189 (24.01%) in 2020, and 298 (37.87%) in 2021. Of these 787 children, 646 (82.08%) patients were included as study participants, and the other patients (17.92%) were excluded because they met the exclusion criteria (**Figure 1**). In 2019, 251 children were enrolled; 160 (63.75%) were male, and 167 (66.53%) were aged 1 to <6 months, 84 (33.47%) were aged 6 to <12 months. In 2020, 170 children were enrolled; 68.24% (116/170) were male, and 99 (58.24%) were aged 1 to <6 months, 71 (41.76%) were aged 6 to <12 months. In 2021, 225 children were enrolled; 160 (71.11%) were male, and 140 (62.22%) were aged 1 to <6 months, 85 (37.78%) were aged 6 to <12 months.

Etiology

Predominant pathogens in the 3-year study

In 2019, the detection rate of at least one pathogen was 56.57% (142/251); a single pathogen was detected in 49.40%

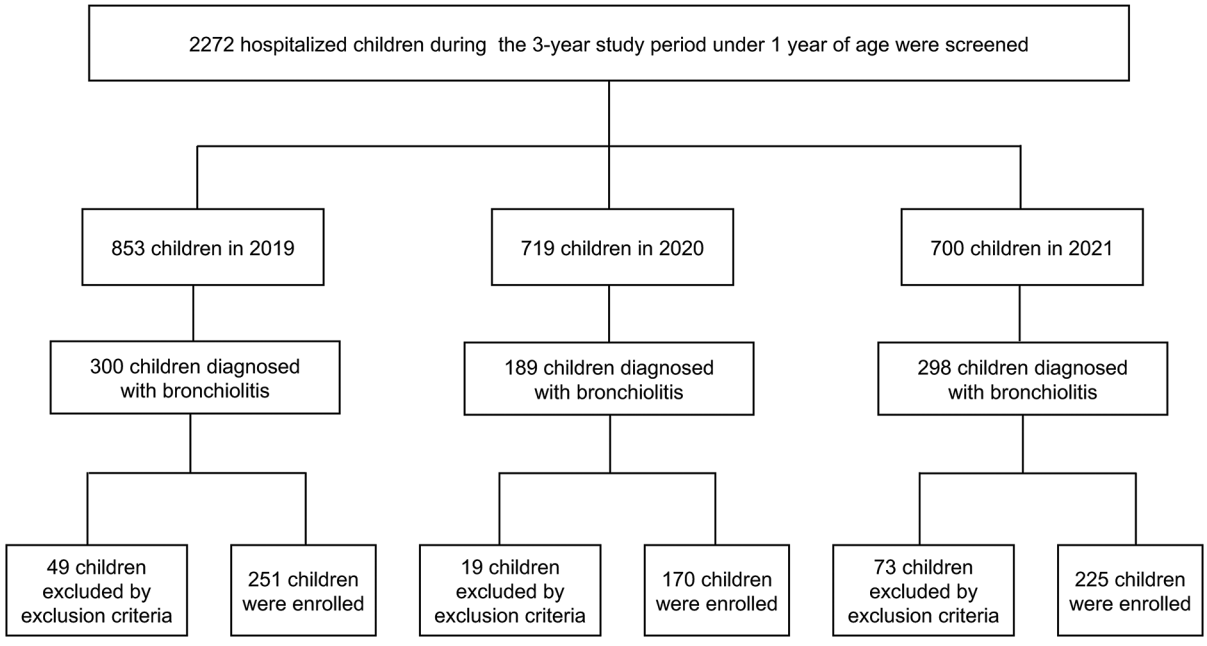

FIGURE 1
Study flow chart.

(124/251) of the cases, and mixed infections were observed in 7.17% (18/251) of the cases. In 2020, the pathogen detection rate was 45.29% (77/170); a single pathogen found in 40% (68/170) of the cases, and multiple pathogens were detected in 5.29% (9/170) of the cases. In 2021, the positive detection rate was 73.78% (166/225); a single pathogen was observed in 60.89% (137/225) of the cases, and mixed pathogens were found in 12.89% (29/225) (**Figure 2**). The pathogen detection rate in 2021 was higher than other groups, and the difference between these rates was statistically significant ($\chi^2 = 34.187$, $p < 0.001$).

In 2019, the most common pathogen was RSV, with a positive detection rate of 33.47% (84/251). This was followed by MP at 11.95% (30/251), HBoV at 7.57% (19/251), HRV at 4.38% (11/251), PIV III at 3.59% (9/251), and hMPV at 2.39% (6/251). RSV was also the most common pathogen in 2020, with a positive rate of 15.29% (26/170). The second most common pathogen was HRV at 12.94% (22/170), followed by HBoV at 7.65% (13/170), MP at 5.29% (9/170), PIV III at 4.12% (7/170), and hMPV at 3.53% (6/170). In 2021, RSV was again the most common pathogen, with 110 (48.89%) children infected. This was followed by HRV at 16.44% (37/225), PIV III at 5.33% (12/225), hMPV at 5.33% (12/225), HBoV at 3.56% (8/225), and MP at 1.78% (4/225) (**Table 1**).

The positive detection rate of RSV was lowest in 2020 and highest in 2021. The rate of MP detection in 2021 was less than that in 2019 but similar to that in 2020. The rate of HRV detection in 2021 was more than that in 2019 but similar to that in 2020. There were no statistical differences in the positive rates over the 3-year study period for hMPV, HBoV, or PIV III.

Patterns of RSV-bronchiolitis over the 3-year study

Because RSV was the most common pathogen in each of the 3 study years, and the detection rate of this virus was markedly different between years, we further compared the positive detection rate of RSV among different age groups as well as the monthly distribution of RSV-positive cases among all age groups and in each age group among the 3 years.

In 2019, the positive detection rate of RSV was 39.52% (66/167) in children aged 1 to <6 months, 21.43% (18/84) in those aged 6 to <12 months. In 2020, the positive detection rate of RSV was 18.18% (18/99) in children aged 1 to <6 months, 11.27% (8/71) in those aged 6 to <12 months. In 2021, the positive detection rate of RSV was 51.43% (72/140) in children aged 1 to <6 months, 44.71% (38/85) in those aged 6 to <12 months (**Figure 3**). In children aged 1 to <6 months, the RSV-positive rate in 2020 was lower compared with those in 2019 and 2021 ($\chi^2 = 27.237$, $p < 0.001$). RSV-positive rate in children aged 6 to <12 months rose in 2021 compared to the previous 2 years ($\chi^2 = 23.932$, $p < 0.001$).

Regarding the monthly distribution over the 3-year study of RSV-positive cases for different age groups, we found that in 2021, the number of positive cases of RSV in all age groups

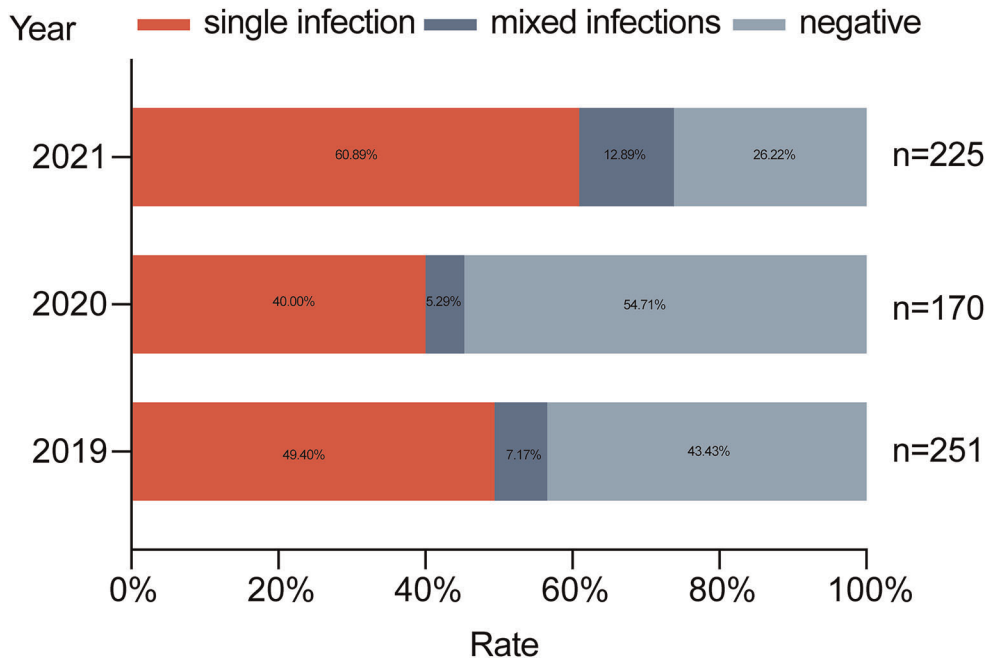

FIGURE 2
Pathogen-detection rates over the 3-year study.

TABLE 1 Predominant pathogens over the 3-year study.

	RSV[a,b,c]	MP[c]	HRV[a,c]	hMPV[d]	HBoV[d]	PIV III[d]
2019	84 (33.47)	30 (11.95)	11 (4.38)	6 (2.39)	19 (7.57)	9 (3.59)
2020	26 (15.29)	9 (5.29)	22 (12.94)	6 (3.53)	13 (7.65)	7 (4.12)
2021	110 (48.89)	4 (1.78)	37 (16.44)	12 (5.33)	8 (3.56)	12 (5.33)
χ^2	48.728	20.456	18.924	2.895	4.132	0.900
P value	0.000	0.000	0.000	0.235	0.127	0.638

Data are presented as *n* (%). RSV, respiratory syncytial virus; MP, Mycoplasma pneumoniae; HRV, human rhinovirus; hMPV, human metapneumovirus; HBoV, human bocavirus; ADV, adenovirus; and PIV III, parainfluenza 3.
[a]Significant difference was observed in the pathogen-detection rate among children in 2019 and 2020.
[b]Significant difference was observed in the pathogen-detection rate among children in 2020 and 2021.
[c]Significant difference was observed in the pathogen-detection rate among children in 2019 and 2021.
[d]No significant difference was observed in the pathogen-detection rate during the 3-year study period.

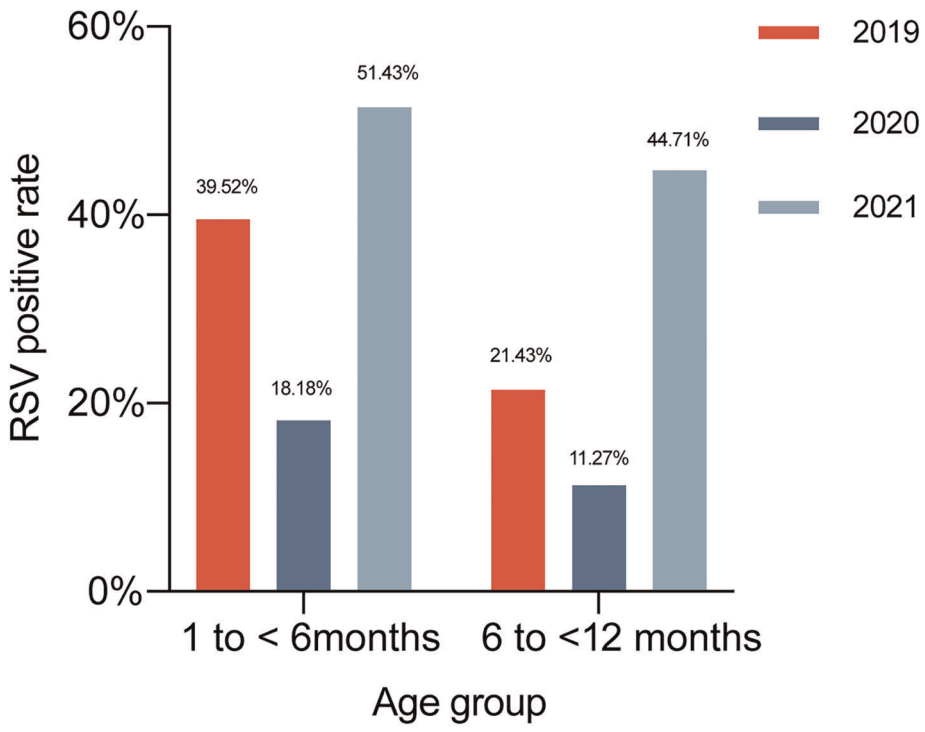

FIGURE 3
The positive RSV detection rates over the 3-year study of different age groups.

as well as in each age group were increased significantly during the winter, and this increase started as early as October.

Finally, we compared the monthly distributions of bronchiolitis cases and the RSV-positive rate over the 3 study years (Figure 5). Longitudinally, the epidemic seasons for bronchiolitis and RSV peaked in the winter months. We found that the number of bronchiolitis cases and the positive detection rate of RSV both dropped sharply beginning in February 2020 when the lockdown measures were initiated. This was followed by increases in the winter of 2021.

Comparisons of the demographic and clinical characteristics of the patients enrolled in the 3-year study

The demographic and clinical characteristics of patients with bronchiolitis are shown in Table 2. There were no significant differences in patient gender among the study years. The median of age, pre-admission symptom duration and length of stay were neither shortened nor extended over the study period. Regarding the clinical manifestations, the proportion of children presenting with stuffy nose rhinorrhea

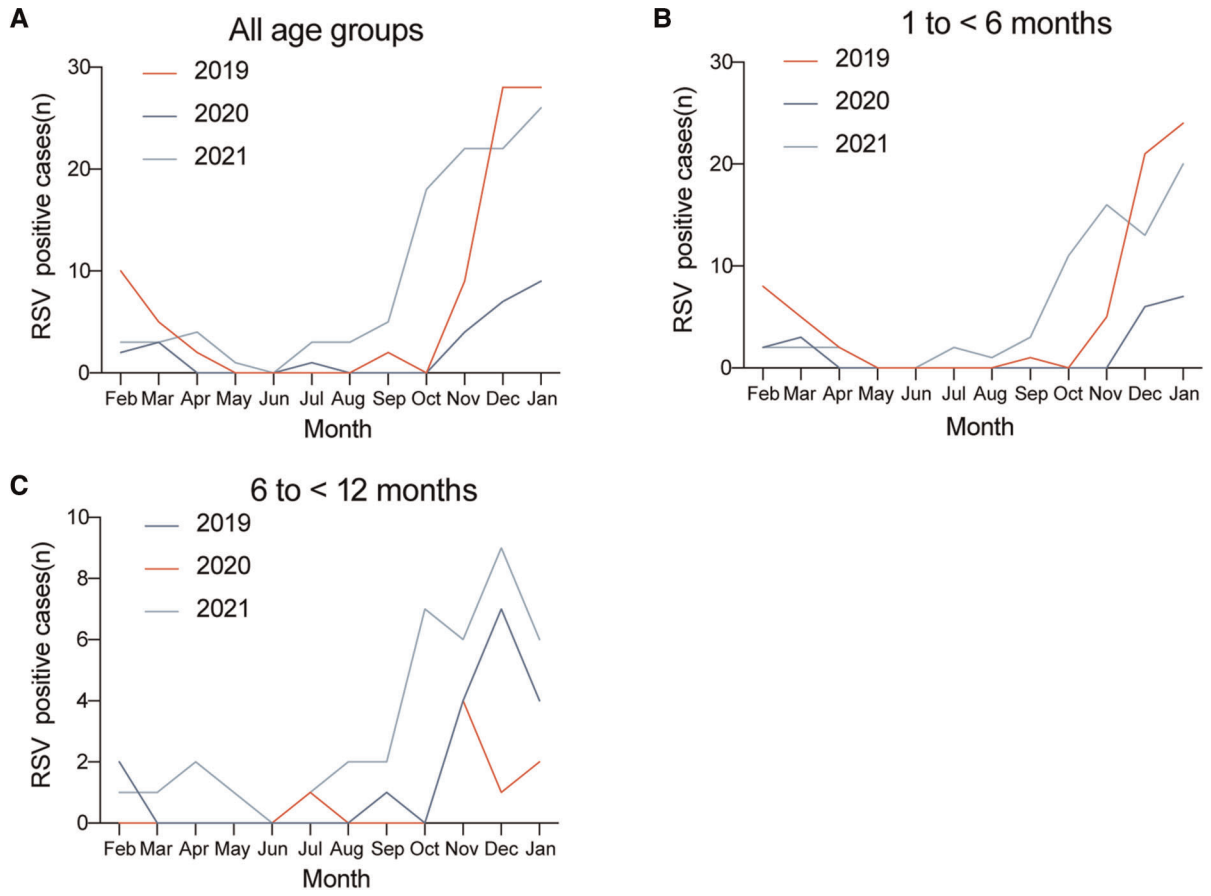

FIGURE 4
Monthly distribution of RSV-positive cases in (A) all age groups, (B) 1 to <6 months, and (C) 6 to <12 months over the 3-year study period.

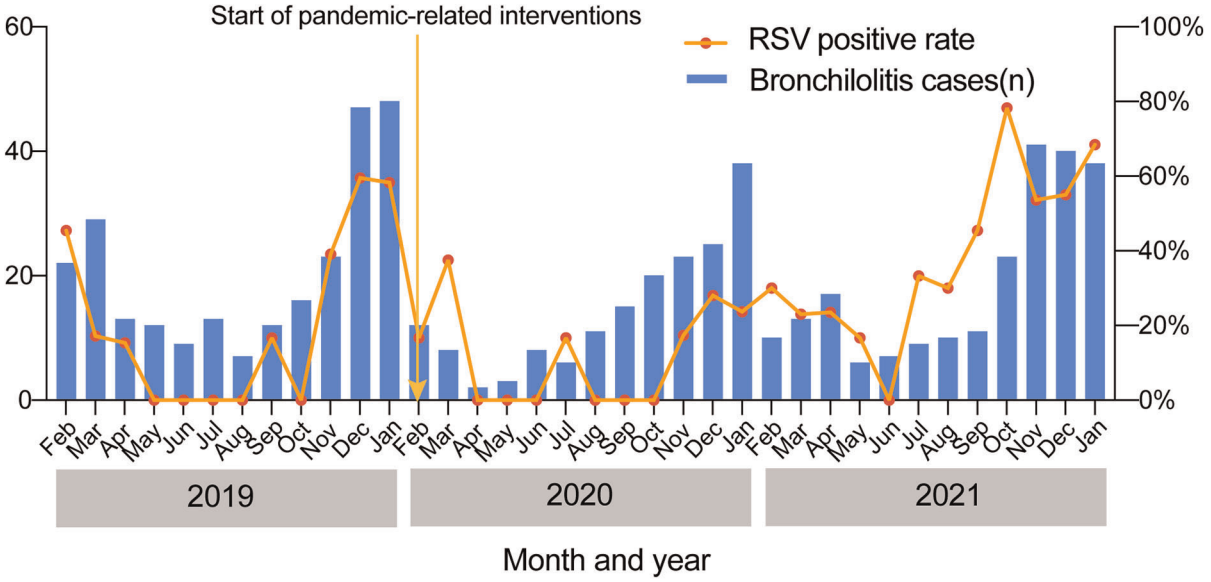

FIGURE 5
Seasonal distributions of bronchiolitis cases and the positive RSV detection rate over the 3-year study.

TABLE 2 Demographic and clinical characteristics of the patients in all age groups over the 3-year study.

Clinical features	2019	2020	2021	*P* value
No. of patients	251	170	225	-
General features				
Gender (male/female)	160/91	116/54	160/65	0.224
Age (months) (IQR)	4.20 (2.33–6.90)	5.01 (2.81–8.10)	5.07 (3.00–7.40)	0.060
Symptom duration prior to admission (day) (IQR)	6.00 (4.00–10.00)	6.00 (4.00–10.00)	5.00 (4.00–9.00)	0.458
Length of stay (day) (IQR)	7.00 (6.00–8.00)	7.00 (6.00–8.00)	7.00 (6.00–9.00)	0.125
Clinic presentation				
Fever [*n* (%)][c]	77 (30.68)	59 (34.71)	65 (28.89)	0.462
Stuffy nose rhinorrhea [*n* (%)][a,b]	122 (48.61)	97 (57.06)	165 (73.33)	0.000
Dyspnea [*n* (%)][c]	17 (6.77)	6 (3.53)	6 (2.67)	0.076
Gastrointestinal symptoms [*n* (%)][b]	64 (25.50)	29 (17.06)	26 (11.56)	0.000
Tachypnoea [*n* (%)][c]	42 (16.73)	25 (14.71)	21 (9.33)	0.056
Cyanosis [*n* (%)][c]	4 (1.59)	3 (1.76)	0 (0.00)	0.100
Laboratory tests				
WBC count (*10^9/L) (IQR)	9.79 (7.45–13.21)	9.77 (7.92–11.92)	9.66 (6.66–13.03)	0.500
Percentage of neutrophils (IQR)	25.20 (16.70–39.20)	27.75 (18.70–42.83)	27.45 (18.10–39.25)	0.415
CRP count >8 mg/L [*n* (%)]	37 (14.74)	27 (15.88)	38 (16.89)	0.813

[a]Significant difference was observed in the clinical characteristic among children in 2020 and 2021.
[b]Significant difference was observed in the clinical characteristic among children in 2019 and 2021.
[c]No significant difference was observed in the clinical characteristic during the 3-year study period.

in 2021 was greater than that in 2019 and 2020, while the proportion of children with gastrointestinal symptoms in 2021 was smaller than that in 2019 but similar to that in 2020. The proportions of children presenting with fever, dyspnea, tachypnea, and cyanosis were similar between years. Moreover, there were no significant differences among the years in the patient laboratory findings, including the white blood cell count, neutrophil percentage, and CRP level.

We also compared the demographic and clinical characteristics among study years within each of the different age groups (**Supplementary Tables S1, S2**). The proportion of 1 to <6 months children presenting with stuffy nose rhinorrhea in 2021 was greater than those in the previous 2 years. The proportion of 6 to <12 months children presenting with stuffy nose rhinorrhea in 2021 was greater than that in 2019 but similar to that in 2020, whereas the proportion of such children with gastrointestinal symptoms in 2021 was smaller than that in 2019 but similar to that in 2020.Consistent with the observations for the entire study population, there were no significant differences in laboratory test results.

Discussion

COVID-19, a coronavirus disease caused by SARS-CoV-2, broke out in Wuhan, China in December 2019 (8). SARS-CoV-2 is a highly contagious respiratory virus that spreads from person to person through contact with respiratory droplets and aerosols from infected individuals (11). In late January 2020, the Chinese government issued a national public health intervention policy, which included closing schools and other public places, prohibiting people from gathering, and encouraging mask wearing, hand washing, and disinfecting, to help prevent the spread of the epidemic. These measures also effectively suppressed the transmission of pathogens that cause bronchiolitis because the transmission pattern of bronchiolitis-causing pathogens, in particular RSV, is similar to that of SARS-CoV-2 (12). However, with the resumption of work and school, the epidemic prevention and control measures have been gradually relaxed.

In this study, we conducted a retrospective investigation of children hospitalized with bronchiolitis over 3 consecutive years (2019, 2020, and 2021) from February 1 to January 31, to compare the clinical features and etiological composition of this population before and during the COVID-19 pandemic. We found that the pathogen detection rate was the lowest in 2020, likely because 2020 was the first year of the COVID-19 pandemic and the associated interventions also reduced the spread of other respiratory pathogens. However, the pathogen detection rate in 2021 was the highest among the 3 study years, possibly because the COVID-19 control measures were eased in the second year of the COVID-19 pandemic.

In this 3-year study, we found that RSV was the predominant pathogen, a finding which is in agreement with many previous studies. Recently, a systematic review and

meta-analysis of the prevalence of common respiratory viruses in children under 2 years of age with bronchiolitis in the pre-COVID-19 pandemic era found that RSV was the dominant etiologic agent of bronchiolitis (13). Correspondingly, the RSV detection rate changed in parallel with the total detection rate over our study years. In the first year of the COVID-19 pandemic, the RSV detection rate was the lowest, which is consistent with findings from previous work (14, 15). In the most severe months of the COVID-19 pandemic in 2020, there was a high uptake of enhanced hygiene and physical distancing measures. Handwashing can damage the lipid envelope that surrounds RSV, thereby impairing its ability to infect host cells (16). Another potential explanation for this difference is that there may have been a change in the ecology of respiratory viruses during the beginning of the COVID-19 pandemic. It is thought that rhinoviruses can stimulate the antiviral defenses of the mucous membrane of the respiratory tract, interfering with and blocking infection by other viruses (17). However, in the second year of the COVID-19 pandemic, the RSV detection rate increased significantly. Seasonal distribution studies conducted on participants in different age groups also found a significant increase in RSV-positive cases during the winter of 2021, and this increase began as early as October. In 2020, researchers from Princeton University and the National Institutes of Health predicted that a decline in common respiratory pathogens, such as RSV and influenza, could increase the population's susceptibility to these diseases, leading to future pandemics when they flare up again. They also predicted that RSV infections would be delayed after the end of the non-pharmaceutical interventions (NPIs) phase of the COVID-19 pandemic response, with a peak of RSV cases expected in many places during the winter of 2021–2022 (18). Our results are in line with their expectations, but this pattern is not unique to China; other regions, including Australia and Tokyo, had already observed an RSV resurgence in September 2020 and July 2021, respectively (19, 20). The timing of RSV prevalence is inconsistent in different regions, which may be related to regional and climate differences. Similarly, we also found that the number of bronchiolitis cases and the positive RSV detection rate dropped sharply beginning in February 2020 when the lockdown measures were taken and then increased again when the intervening measures were relaxed, especially in the winter of 2021. The resurgence of RSV may be attributed to "immune debt", a term established to characterize the lack of protective immunity resulting from prolonged periods of low exposure to a certain pathogen, making a larger percentage of the population susceptible to the disease (21). Due to the transitory immunity established by RSV through virus exposure, maternal antibodies deplete quickly, and without seasonal exposure, immunity declines and susceptibility to subsequent and potentially more severe infections rises (22).

The positive rate of MP detection in 2021 was lower than that in 2019 but similar to that in 2020. This result is similar to those from a study conducted in Chengdu, China. In that study, researchers analyzed the prevalence of MP infection in children with respiratory symptoms from January 2017 to December 2020, and they found that public health measures adopted during the COVID-19 pandemic response could effectively control the spread of MP (23). MP, which is the smallest independent pathogenic microorganism between bacteria and viruses, often affects school-aged children. It continues to circulate and spread periodically, exhibiting an epidemic peak every 3 to 7 years.MP is a common causative pathogen of children's community-acquired pneumonia and, like RSV, is an important causative pathogen of bronchiolitis. However, in our study, the prevalence of MP was not found to rebound like that of RSV. It is unclear whether this occurrence is because of the epidemiological characteristics of MP itself or a low susceptibility to MP in children under 1 year of age.

Our study found that the detectable rate of HRV in 2021 was higher than that in 2019 but similar to that in 2020. Rhinoviruses are non-enveloped organisms that remain on environmental surfaces longer than many other pathogens (24), and they are not sensitive to ethanol-based disinfectants (25). So it is possible that these factors contributed to the rise in the positive rate of rhinovirus, despite the widespread use of disinfectants during the pandemic. However, the precise mechanism is still unknown.

We found that, except for differences in the percentage of patients with stuffy nose rhinorrhea, and gastrointestinal symptoms, there were no significant differences in the prevalence of bronchiolitis clinical features over the 3-year study period. The increase in the percentage of patients with bronchiolitis who reported stuffy nose rhinorrhea during the COVID-19 pandemic from that in 2019 may be due to the increased detection rate of rhinovirus, given that the most common symptoms of HRV infection are runny nose, nasal congestion, sore throat, and cough. During the COVID-19 pandemic, the percentage of patients with bronchiolitis who reported gastrointestinal symptoms (vomiting/diarrhea) decreased, possibly owing to the small sample size and the COVID-19 intervention measures.

Our study has some limitations. First, we included only patients with bronchiolitis who had undergone nasopharyngeal secretion examinations, and we did not enroll any patients with only a bacterial infection. Second, our samples were all collected from Suzhou City; thus, they do not fully reflect the epidemiological changes owing to geographical limitations. Finally, all of the children enrolled in our study were inpatients because it was not possible to obtain the epidemiology of respiratory pathogens from outpatients.

Conclusion

After the implementation of interventions to tackle the spread of SARS-CoV-2 infection, we observed a significant decline in the pathogen detection rate and RSV-positive rate in the first year of the COVID-19 pandemic. A rebound appeared to take place in the second year of the COVID-19 pandemic. The detection rate of MP decreased while the positive rate of HRV increased during COVID-19 pandemic as compared with those rates before the pandemic. The difference in pathogen composition also led to an observable difference in the clinical characteristics of children with bronchiolitis before and during the epidemic. At present, the global COVID-19 pandemic remains serious. Given that the impact of the relaxation of preventative measures against COVID-19 on the diseases caused by other respiratory viruses, including RSV, in the susceptible population is unknown (26–28), it is of great importance to strengthen disease prevention, control, and monitoring systems.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of the Children's Hospital of Soochow University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

XJ and TW wrote the main manuscript text. YY and ZC conceptualized and designed the study. GD and HS collected the clinical data. WJ contributed to the statistical analysis and interpretation of data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.974769/full#supplementary-material.

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SARS-CoV-2 viral clearance and viral load kinetics in young children (1–6 years) compared to adults: Results of a longitudinal study in Germany

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Objective: To investigate SARS-COV-2 viral clearance and viral load kinetics in the course of infection in children aged 1–6 years in comparison with adults.
Methods: Prospective cohort study of infected daycare children and staff and their close contacts in households from 11/2020 to 06/2021. Adult participants took upper respiratory tract specimen from themselves and/or their children, for PCR tests on SARS-CoV-2. Data on symptoms and exposure were used to determine the date of probable infection for each participant. We determined (a) viral clearance, and (b) viral load dynamics over time. Samples were taken from day 4–6 to day 16–18 after diagnosis of the index case in the respective daycare group (5 samples per participant).
Results: We included 40 children (1–6 years) and 67 adults (18–77 years) with SARS-CoV-2 infection. Samples were available at a mean of 4.3 points of time per participant. Among the participants, the 12-day study period fell in different periods within the individual course of infection, ranging from day 5–17 to day 15–26 after assumed infection.

Children reached viral clearance at a median of 20 days after assumed infection (95% CI 17–21 days, Kaplan-Meier Analysis), adults at 23 days (95% CI 20–25 days, difference not significant). In both children and adults, viral load decreased over time with trajectories of the mean viral load not being statistically different between groups. Kaplan-Meier calculations show that from day 15 (95% CI 13–15), 50% of all participants had a viral load <1 million copies/ml, i.e. were no longer infectious or negative.
Conclusion: Children aged 1–6 and adults infected with SARS-CoV-2 (wild type and Alpha variant) did not differ significantly in terms of viral load kinetics and time needed to clear the virus. Therefore, containment measures are important also in the daycare settings as long as the pandemic continues.

KEYWORDS

viral clearance, viral load, daycare center, viral shedding, COVID - 19, infectiousness children, SARS-CoV-2 children, infectivity children

Introduction

The role of daycare children in the spread of SARS-CoV-2 has been discussed controversially since the beginning of the COVID-19 pandemic in March 2020. Daycare centers promote the physical and psycho-social development, health and well-being of young children, and in many European countries reach a high proportion of young children from all social groups. In Germany, for example, 35% of 0- to 2-year-olds and 93% of 3- to 6-year-olds are enrolled in a daycare program (1). Pandemic-related closures or (repeated) quarantine and isolation periods of children are likely to have detrimental effects on psychosocial well-being, physical activity and body weight (2–5). However, daycare programs mainly serve children in an age group that is (for the time being, June 2022) not eligible for vaccination against SARS-CoV-2 in Germany. In addition, measures that help contain the transmission of SARS-CoV-2, such as wearing masks and physical distancing, are difficult to implement among toddlers and preschoolers. Therefore, understanding the transmissibility and kinetics of SARS-CoV-2 among daycare children is critical for the development of adequate mitigation policies, such as recommended time for isolation, testing strategies, and hygiene concepts contributes to understanding the role of daycare children in the spread of SARS-CoV-2 also in comparison to school children and adults.

Two indicators that help assess the infectivity of an individual and the duration of infectiousness are viral clearance (VC) and viral load (VL). VC is defined as the state when the virus is eliminated from the respiratory tract, as can be seen in negative PCR tests after one or more positive PCR test(s). VL refers to the amount of viral RNA detected in specimen from the respiratory tract. As we have learned in the course of the pandemic with regard to SARS-CoV-2, a positive PCR result does not equate to infectivity of the person tested. Rather, a threshold of SARS-CoV-2 RNA copies/ml has been determined above which it can be assumed that the amount of viral material is sufficient for transmission. Therefore, the measurement of viral load is helpful to better interpret PCR test results. Studies from the early period of the COVID-19 pandemic from Germany and the USA did not find significant differences between the VL of adults as compared to children aged 1–5 or 1–6 years, respectively (6, 7).

As for viral clearance, a comprehensive systematic review and meta-analysis, including studies published until 6/2020, yields a mean period of 17 days with a maximum of 83 days needed for COVID-19 patients of all age groups to reach VC from SARS-CoV-2 in the upper respiratory tract (8).

Most studies on VC and VL are based on data of hospitalized individuals or individuals seeking medical attendance (9–12). Mild or asymptomatic cases are probably underrepresented in these studies. This may especially be true for young children, as COVID-19 in childhood often does not present with any (severe) symptoms. We see a dearth of studies that (a) investigate the infectivity of SARS-CoV-2 positive individuals over time in a non-clinical community setting, (b) that put the PCR test results in relation to the PCR test results in the individual time course of infection, and (c) explicitly focus on the age group of daycare children. Analyses have confirmed transmissions from infected children to close contacts within the daycare setting, and also to household members (13), highlighting the importance of investigating the role of daycare children in the infection process in more detail.

Therefore, we will examine the following research questions: How long does it take for children aged 1–6 to clear SARS-CoV-2 from their upper respiratory tract (VC)? Is there a difference to adults? And how does the viral load (VL) of SARS-CoV-2 develop over time in children aged 1–6, compared to adults?

Materials and methods

Study design/subjects

We analyzed data from the COALA study (14), which is an outbreak-related examination study in daycare centers, carried out from October 2020 to June 2021 in the "second" and "third wave" of the pandemic in Germany. At this time SARS-CoV-2 wildtype, followed by the alpha variant of concern (VOC), were predominant. COALA has a prospective longitudinal, case-related study design. Daycare center groups with one or more SARS-CoV-2 positive cases (child or staff) were enrolled in the study. 30 daycare groups from 20 different communities (in 9/16 federal states) all over Germany were included. SARS-CoV-2 cases as well as their close contacts within their respective daycare group and household were visited at home by a trained study team four to six days after the SARS-CoV-2 test date of the index case (i.e., the person who first tested positive in the outbreak). Further details of the study design and methods are described elsewhere (14).

A total of 1,047 individuals was included in the COALA study ($n = 447$ children and $n = 600$ adults), of which only positively tested individuals, were included in the current analyses. Amongst the children, $n = 343$ were in the age group of daycare children (1–6 years of age; children of older ages were siblings and not included here).

Sampling

During the home visits, mouth-nose swabs (MNS) and saliva samples were taken. For the following 12 days after the

home visit, participants were instructed to take these samples from their upper respiratory tract and from their children themselves every three days (self-sampling) and to send them to the Robert Koch Institute (RKI) by postal shipment (**Figure 1**).

The frequent sampling and PCR testing over the 12 days study period made it possible to detect newly infected participants at an early stage. Moreover, it allowed to determine viral clearance and to quantify viral loads for each participant over the course of infection.

Assessment of symptoms

After the initial surveys during the home visits, household members were instructed to record clinical symptoms throughout the 12 days study period in a standardized symptom-diary, documenting symptoms e.g., fever ≥38 °C, chills, cough, shortness of breath.

Laboratory testing

All biological samples (MNS and saliva) were tested for SARS-CoV-2 RNA presence and quantity by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) as described by Michel et al. (15). If volume for saliva samples was insufficient (<100 µl) PBS was added to reach a volume of 300 µl. Results were given in cycle threshold (CT) values (number of needed cycles surpassing the threshold for a positive test). For better comparison with other studies, CT values were converted to actual RNA copy numbers per milliliter. As a positive PCR result (values >=10^1 RNA copies/ml; ≙ CT 40 in our laboratory) does not equate infectivity, a threshold within the positive PCR range was defined above which an individual's infectivity can be assumed. For SARS-CoV-2, this threshold was set at 10^6 RNA copies/ml (≙ CT 24.7 in our laboratory) (16). Log10 RNA copies/ml were calculated based on the cycle threshold (CT) of the E gene. Comparative analysis of E gene and delta CT of E gene and c-myc, either obtained in the multiplex or in a single plex PCR, showed no significant difference in exemplary sample courses, allowing the use of the E gene CTs directly for calculation of RNA quantities.

Determination of assumed infection date

In order to determine the starting point for calculating the period until viral clearance is reached, we decided against using the onset of symptoms (as young children are often asymptomatic or present only with very unspecific symptoms). Neither did it seem recommendable to use the date of the first positive PCR test, as the timepoint of testing among the cases differed substantially (e.g., sometimes asymptomatic primary cases were not detected before secondary cases who presented with symptoms were diagnosed). We therefore decided to determine the probable date of infection with SARS-CoV-2 for each participant, analyzing comprehensive data from questionnaires and lab results: date of first positive PCR test, result of antibody tests, symptom onset of cases and their respective contact persons, exposure to other infected individuals in and outside the daycare group. Reconstructing the probable infection dates

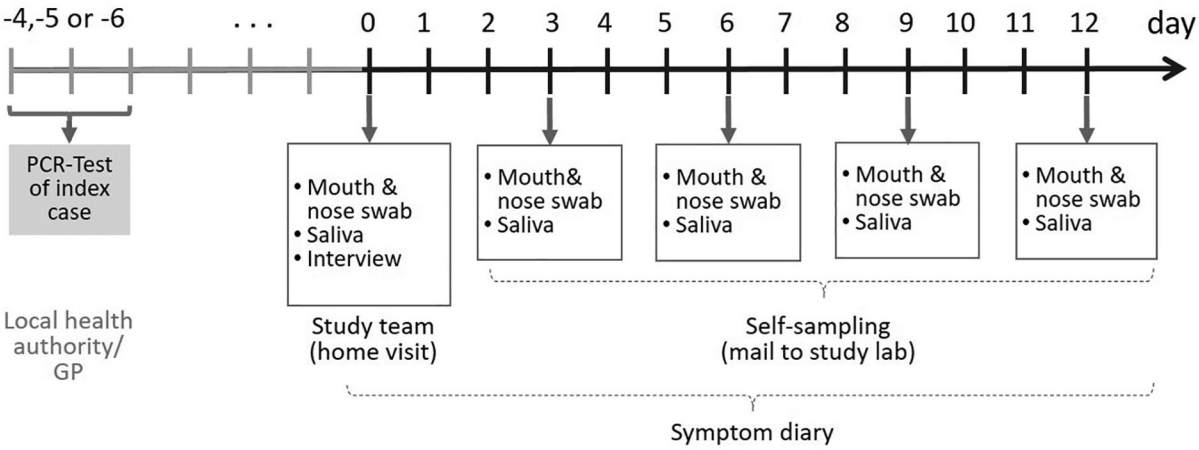

FIGURE 1
Timeline sampling scheme for bio samples taken from the participants of the COALA study (SARS-CoV-2 index cases, secondary cases and close contacts of SARS-CoV2 cases in the respective daycare center group and households). Participants were enrolled 4–6 days after the index case got tested. GP = general practitioner.

also made it possible to determine the probable primary cases of each outbreak (13). As a consequence, the study period of twelve days was set within a different time frame in each participant's individual course of infection: e.g., for one participant (e.g., asymptomatic primary case), the first sampling of the study may have taken place on day 8 after probable date of infection and then continued until day 20 of his or her infection course. For another participant (e.g., secondary case), the study period may have started at the very date of infection and then lasted until day 12 of his or her individual infection course.

All sampling days were then expressed as days since assumed infection for each participant. For calculations, the index cases' first positive test date by the health department and a mean incubation period of SARS-CoV-2 of six days were taken as a basis and applied to index cases and secondary cases in daycare centers and households.

Definition of viral clearance

To determine viral clearance, we calculated the time from the assumed date of infection until the first negative PCR test, which was not followed by another positive PCR test in the self-sampling. If only one of the two samples received (MNS or saliva) was positive, the person was classified as positive at that time.

Definition of viral load

Viral load is defined as the number of SARS-CoV-2 RNA copies/mL in the PCR sample. If both the MNS and the saliva sample of a person had a positive result on the same day, we used the sample with the higher viral load for the analyses.

For the analysis of viral load, CT values measured in the PCR analysis were converted into RNA copies/ml by the following formula: 83.33333 * e^ [(CT value – 38.248)/–1.4] that was determined by serial dilution and measurement of pre-quantified target RNA. Then, the number of RNA copies/ml was transformed to the log scale (with base 10), resulting in a linear transformation of the original CT values, but with opposite sign (15).

Statistical analysis

To examine the time to viral clearance, Kaplan-Meier survival time analysis was applied. The time from infection to the first negative test is equaled with "survival" time. A Kaplan-Meier analysis computes the probability of occurrence of a negative PCR-test at a certain point of time, taking into account the number of participants still in the study at this time. For some participants, a negative test was not yet recorded during the study period, i.e., they still had a positive test on the last sampling day. In these cases, the event of interest (PCR negativity) did not happen during this time. These participants were labeled as "censored observations". For these censored observations, the only information is that the event (=first negative test) did not occur by the end of the study, which can be due to the following reasons: (a) inclusion in the study early in the individual course of infection, e.g., when a secondary household case got infected during the last days of the study period, (b) early study dropouts and (c) prolonged viral shedding.

To test for significant differences between daycare children and adults, a log-rank test was applied, which is the standard test for comparison of two groups in Kaplan-Meier survival time analysis.

For viral load over time, mean, median, quartiles and distribution were calculated separately for children and adults. The difference in the mean course of viral load for children vs. adults is given as mean (**Figure 4**) and median with 95%-confidence interval (**Figure 5**) for each time point. The difference was tested for significance in a linear mixed model which included VL as dependent variable, the individual participant as random effect and day since assumed infection (as a linear variable), age group (child/adult) and specimen type (mouth-nose swab or saliva) as independent variables. Descriptive calculations were performed using STATA 17.0 (Stata Corp, College Station, Texas, USA, 2021). SAS 9.4/TS1M7 (SAS Institute Inc., Cary, NC, USA, 2016) was used for survival analysis and mixed model calculations and R 4.1 (R Foundation for Statistical Computing, Vienna, Austria, 2022, www.R-project.org) was used for graphical representation.

Results

Sample

Of all included individuals, 67 adults (18–77 years; female = 56,7%) and 40 children (1–6 years; female = 50%) were tested positive by PCR for SARS-CoV-2 in at least one specimen during the COALA study and were included into the current analysis.

Viral clearance

26/40 children and 29/67 adults reached viral clearance during the study period, with the last sampling occurring on day 8 to 30 (children), and day 5 to 27 (adults), respectively, after their individual infection date. The remaining 14 children and 38 adults still had positive PCR results at the time of the last sampling in the study period. Therefore, no statement on VC can be made for these participants on an

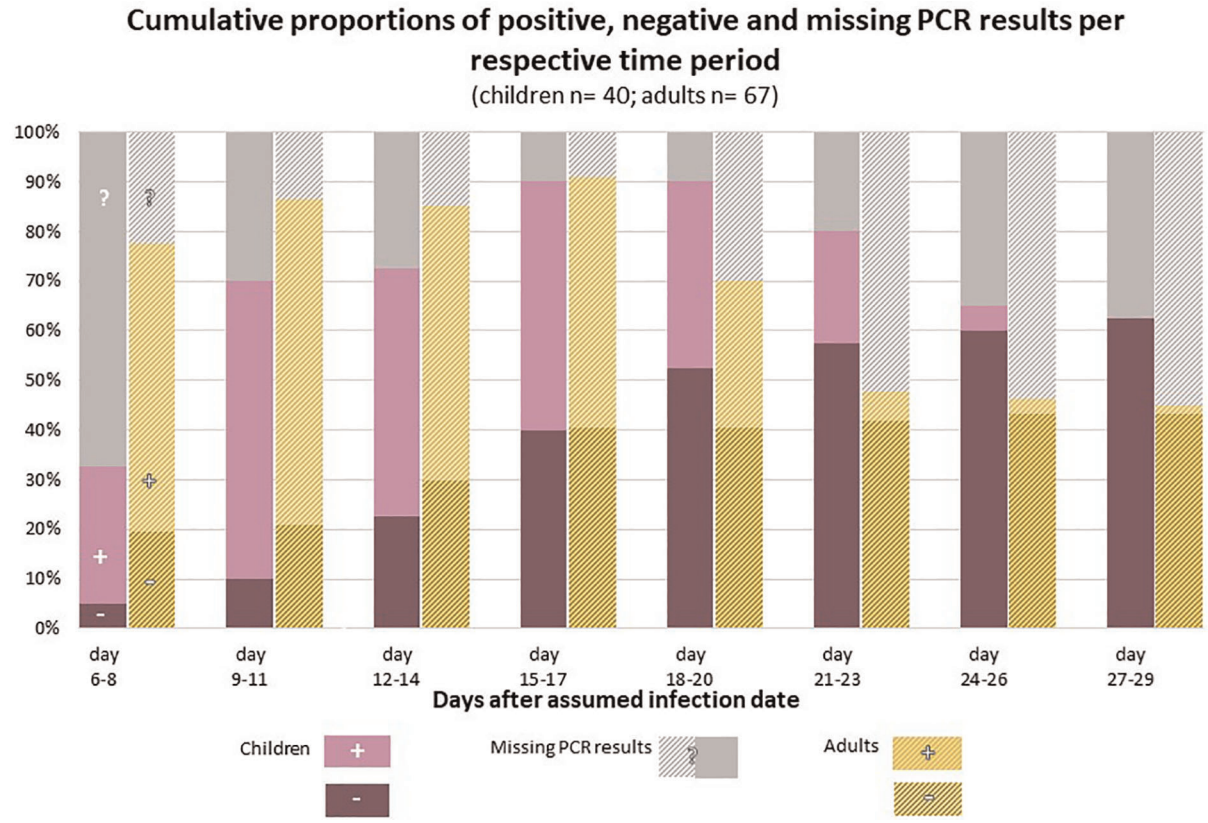

FIGURE 2
Cumulative proportion of children and adults with a negative (purple) PCR test on SARS-CoV-2, compared to the proportion of children and adults who were still tested positive (pink) at different points of time after the assumed infection. PCR test results were not available from all study participants at all points of time; the proportion of children and adults for whom no information of a SARS-CoV-2 PCR was available at a given point of time is shown in grey color. Reasons for missing PCR tests were for example that participants were not yet included in the study, e.g. index cases had probably been infected more than 6–8 days ago when the study team arrived for the testings (enrolment started 4–6 days after test date of index case). Other cases finished their 12-day self-sampling period earlier than day 20, or 24, or 27 after their infection date. In addition, few participants skipped single sampling appointments.

individual basis, but they can be included in the Kaplan-Meier analysis.

Figure 2 shows the cumulative proportion of children and adults who were found to have cleared SARS-CoV-2 at different points of time after infection.

According to Kaplan-Meier-survival time analysis, the estimated median time from infection to the first negative PCR test, i.e., time to viral clearance, is 20 days (95% CI 17–21 days) for the daycare children. 1/4 had their first negative test at or before an estimated 16 days after infection, 3/4 at or before day 22. The percentage of children having reached VC at day 21 was estimated as 71% (95% CI 55%–85%).

In the adult cohort, viral clearance occurred at an estimated median of 23 days after infection (95% CI 20–25 days). 1/4 had their first negative test at or before an estimated 18 days since infection, 3/4 at or before day 25. The percentage of adults having reached VC was 47% by day 21 (95% CI 33%–63%), 56% by day 24 (95% CI 40%–72%) and 90% by day 25 (95% CI 63%–99%) (Figure 3).

The log-rank test for differences between children and adults has a p-value of $p = 0.10$ $[\chi^2(1) = 2.65]$, showing that distributions between the groups do not differ significantly.

The median time for viral clearance for asymptomatic and symptomatic cases was similar (16 days (95% CI 14–18 d.) and 17 days (95% CI 16–20 d.), respectively). Calculating from first positive PCR-test, median VC was 9 days (95% CI 9–10 d.).

Viral load

Changes of viral load over time, throughout the natural course of infection, vary substantially between individuals (Figure 4).

The broad range of all viral load values of different individuals on certain days after infection is also reflected in the boxplot figure (Figure 5).

Regarding the raw mean values of viral load, there is a decreasing trend over time since infection, with no clear

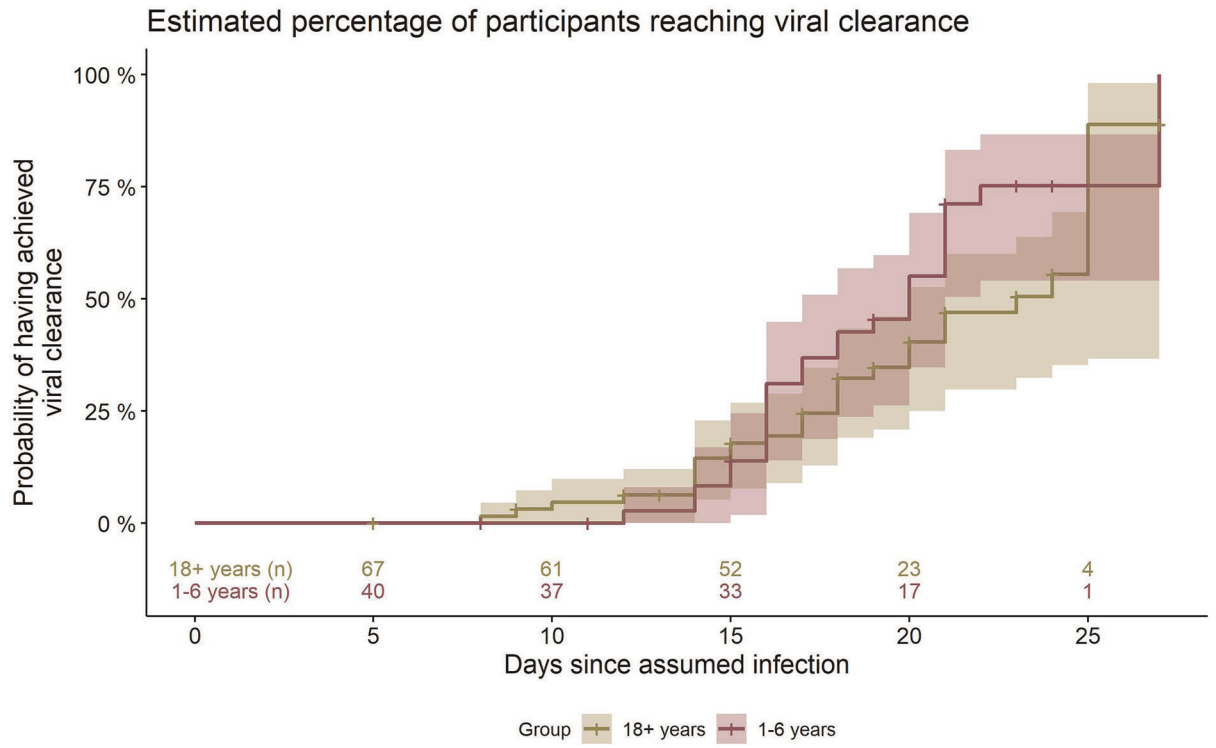

FIGURE 3
Estimated percentage (from Kaplan-Meier survival analysis) of daycare children and adults having accomplished viral clearance at the respective timepoints during the course of infection. Days counted since day of assumed infection (day zero). Group 18+ and 1–6 years: numbers of participants still positive immediately before the respective time point. Shaded area: 95% CI.

differences between children and adults. The mean viral load of both groups (adults and children) stays below the threshold of 10^6 RNA copies/ml, considered necessary for transmission, from day nine the course of infection. The same holds true for the median (**Figure 5**), indicating that at any time point after day nine, less than half of the positive specimens (often, considerably less than half) were above the infectivity threshold. Single values above this threshold were observed until day 18 in children and until day 24 in adults.

Furthermore, the linear mixed model indicates an estimated difference in the trajectories of the mean viral load over time of −0.35 log10 RNA copies/ml (95% CI -0.79-0.10) for children vs. adults ($p = 0.12$), the observed difference is compatible with a random fluctuation.

Among the participants with a positive PCR test result, those whose viral load is considered high enough to allow virus transmission to others, are of special interest from a public health point of view. **Table 1** details the proportions of those tested who can be considered infectious, confirming graphic evidence from **Figures 3** and **4**. The proportion of individuals in whose samples more than 10^6 SARS Co-V-2 RNA copies/ml could be detected, peaked until days 11 and 12, and then decreased markedly (**Table 1**).

Kaplan-Meier calculations show that from day 15 (95% CI 13–15), 50% and from day 17 (95% CI 15–20), 75% of all participants had a VL that can be classified as either negative or no longer infectious (graph not shown).

Overall, more than half ($n = 63/107$, 59%, 95% CI 49%–68%; χ^2-test: $p = 0.32$) of the participants with positive SARS-CoV-2 test did not show a VL considered to be infectious in any of their respective tests during the study period.

Discussion

Summary of main findings

Among daycare children (age 1–6 years), the median time span from assumed SARS-CoV-2 infection to the first negative PCR test was 20 days, which was not significantly different from the age group of adults (23 days). Presuming an incubation period of six days, this may roughly be translated into a median period of 14 days after onset of symptoms (if any) until the virus is cleared from the respiratory tract.

Although SARS-CoV-2 may persist for weeks and viral loads throughout the individual course of infection are very heterogeneous, its quantity in the airways is mostly below the

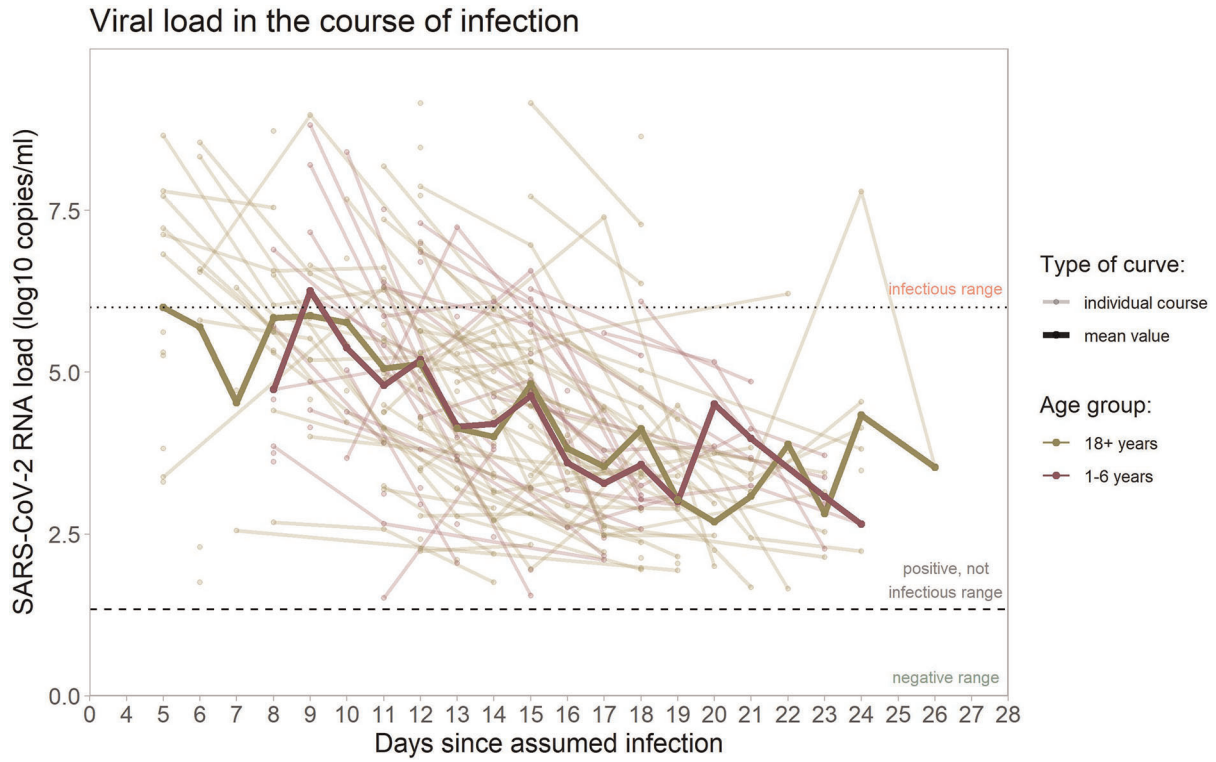

FIGURE 4
SARS-CoV-2 RNA copies/ml in the samples from upper respiratory tract. Viral load courses (individual courses and mean courses of children and adults), shown from the day of assumed infection (day zero) onwards. Dotted line marks 10^6 RNA copies/ml as threshold for infectivity (>=10^6 RNA copies/ml denotes infectious range); dashed line marks threshold for positivity with values >=10^1 RNA copies/ml considered as positive. Analysis restricted to samples considered positive (102 samples in 40 children, 214 samples in 67 adults) with increasingly fewer samples in the progression of the course of infection.

threshold set for an individual's infectivity, as could be shown by tracking of viral load over time. 17 days after assumed infection (i.e., circa 11 days after symptom onset), 75% of participants were not infectious anymore, as they had either a negative PCR test or a positive test with a viral load of less than 1 million copies per milliliter. Viral load, in the mean, decreased gradually over time, and the viral load trajectory did not differ significantly between young children and adults.

Further findings from the COALA study, which focused on actual transmission risk and secondary attack rates (13) also underscore no significant differences between daycare children and adults: In the enrolled daycare centers, the transmission risk from pediatric primary cases did not differ significantly from that from adult primary cases (11% vs. 7.0% of close contacts got infected).

Comparisons with other studies

Our study confirms the finding that SARS-CoV-2 is often present in the upper airways for weeks, while other viruses such as influenza are rarely shed for more than seven days (8, 17–19).

Prolonged periods of continued or inconsistent positive testing may be explained by the physiological half-life of respiratory epithelial cells (three months) and the ability of the PCR tests to detect non-viable viral fragments at very low concentrations (20, 21).

When comparing our results with those of other SARS-CoV-2 studies, it should be reflected that there are different methods to calculate the elimination period. VC, i.e., the state when a person has "cleared" the virus from their body, is sometimes defined as the day of the last positive PCR test, in other studies as the day of the first negative PCR test. For calculating the period until VC is reached, studies have referred to the day of symptom onset, or the day of the first positive PCR test. Therefore, calculations of the period needed for an individual to eliminate the virus can only cautiously be compared between different studies.

Regarding VC we found three relevant studies: Gupta et al. (22) analysed data of 85 children and adolescents (aged 0–18) who were tested positive for SARS-CoV-2 and admitted to hospital during April and May 2020 in Rajasthan/India. The participants were tested by PCR every 3 days. The authors found that median time for children to clear the virus was 7

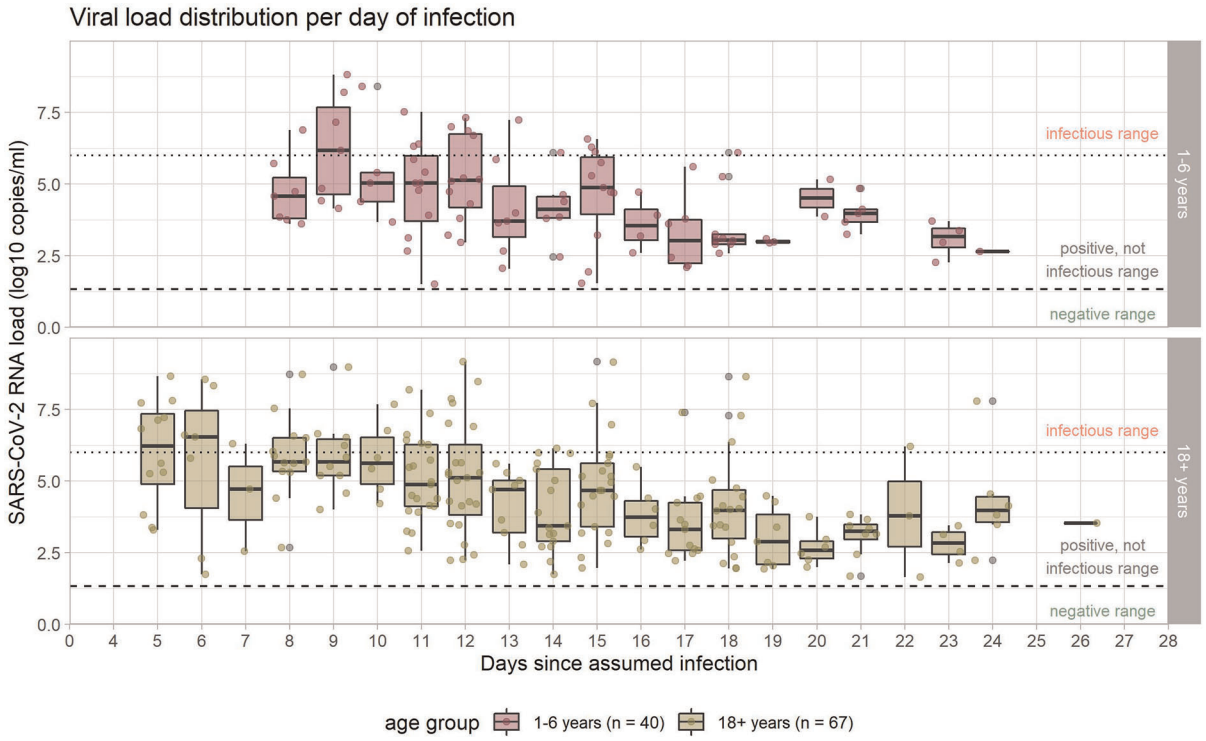

FIGURE 5
Viral load in the course of infection. Medians are calculated for those samples which were available on the given days, and are indicated by midlines; the top and bottom edges of boxes represent the interquartile range (IQR). Whiskers indicate the upper and lower values. Dotted line marks the threshold for infectivity 10^6 RNA copies/ml (≙ CT 24.7 in our laboratory); dashed line marks the threshold for positivity with values >=10^1 RNA copies/ml (≙ CT 40 in our laboratory) considered as positive. Analysis restricted to samples considered positive (104 samples in 40 children, 214 samples in 67 adults) with increasingly fewer samples in the progression of the course of infection.

days, calculated from symptom onset, which is considerably shorter when compared to our results. Gupta et al. found that 99% of children reached viral clearance by day 15 after symptom onset; this may correspond to approximately 19–21 days after infection, a period after which, in our study, only approx. 50%–75% of children were free of virus, according to the Kaplan-Meier analysis.

A German study among 208 participants carried out between January and May 2020 (23) showed results similar to ours. Here, the median time to reach VC was 12.5 days, and 75% of the participants were determined free of SARS-CoV-2 21.5 days after symptom onset. However, the study had not focused on young children, but included participants of all age groups, without reporting on differences between age groups.

A notably longer median time to clear the virus from one's body was found in a study conducted from March-June 2020 in Washington/USA. Median time to reach VC (from first positive PCR test) was 22 days for children aged 0–5 years ($n = 24$) (24). Overall, the elimination period in the different studies shows inconsistent duration of about 13 to 24 days for VC, and our result of 20 days fits well with the current state of research.

Three studies were found that assessed individual VL of SARS-CoV-2 over time: In the sample presented by Bahar et al., there was no meaningful difference between viral loads throughout the course of SARS-CoV-2 infection between children and adults (24), which is similar to our finding. Costa et al. investigated 256 infected children (1–18 years) and 928 adults (from June 2020-January 2021 in Spain) and found similar SARS-CoV-2 viral loads in the first days after symptom onset. However, PCR tests after three days after symptom onset showed a significantly lower VL in children as compared to adults ($p = 0.002$) (25). Our results could not directly confirm this finding, but this may partly be due to the smaller sample size.

Jang et al. also looked into the progression of VL, and describe that from about day 10 post-infection, SARS-CoV-2 RNA can still be detected, but its amount remains predominantly below an infectious threshold (26). This finding is consistent with the viral load dynamics found in our study.

Limitations/strengths

First, it is a limitation that the sample size of the COALA study is relatively small, and may not be representative for all

TABLE 1 Number and proportion of children and adults showing a viral load in the infectious range, detailed for different days of the infection course. Samples are not provided by all participants on each day, because (a) the sampling scheme asked participants to send in swabs every third day only, and (b) the sampling scheme started on day 4–6 after testing date of the index case, therefore participants were included on different days of their individual course of infection, and sampled over a period of 12 days. Dashes: no samples received.

	PARTICIPANTS WITH A VIRAL LOAD ABOVE 10^6 RNA COPIES/ML					
	Children (1–6 years)			Adults (18–77 years)		
DAYS AFTER ASSUMED INFECTION	PCR tests with VL >10^6 RNA copies/ml, *n*	% of all available tests that day (respective *n*)	% of all pediatric participants, *n* = 40	PCR tests with VL >10^6 RNA copies/ml, *n*	% of all available tests that day (respective *n*)	% of all adult participants, *n* = 67
5	-	-	-	6	50.0% (12)	8.8%
6	-	-	-	4	57.1% (7)	5.9%
7	-	-	-	1	33.3% (3)	1.4%
8	1	14.3% (7)	2.5%	4	30.8% (13)	5.9%
9	4	57.1% (7)	10%	4	40.0% (10)	5.9%
10	1	20.0% (5)	2.5%	2	33.3% (6)	2.9%
11	3	25.0% (12)	7.5%	6	28.6% (21)	8.6%
12	4	30.7% (13)	10%	6	26.1% (23)	8.9%
13	1	14.3% (7)	2.5%	0	0% (9)	0%
14	0	0% (6)	0%	1	5.9% (17)	1.5%
15	3	27.2% (11)	7.5%	3	14.3% (21)	4.4%
16	0	0% (5)	0%	0	0% (6)	0%
17	0	0% (6)	0%	1	7.7% (13)	1.5%
18	0	0% (9)	0%	3	16.7% (18)	4.5%
19	0	0% (3)	0%	0	0% (7)	0%
20	0	0% (2)	0%	0	0% (6)	0%
21	0	0% (5)	0%	0	0% (8)	0%
22	-	-	-	1	33.3% (3)	1.5%
23	0	0% (4)	0%	0	0% (5)	0%
24	0	0% (1)	0%	1	16.7% (6)	1.5%

children infected with SARS-CoV-2 in this age group. In addition, the participating adults were recruited from the daycare setting, which means they were mostly staff or parents of young children. On the other hand, this sample can provide insights into courses of SARS-CoV-2 kinetics outside the clinical setting. The participants were closely examined over a period of almost two weeks. Thus, compared to other studies, it was possible to analyze viral load dynamics much more precisely, and to identify SARS-CoV-2 cases that might not have been detected otherwise, for example due to a lack of symptoms.

Second, as our study design focused on the role of children, we included pediatric index cases preferentially to adult index-cases. As sampling started at the same point of time for primary and secondary cases, primary cases were more likely to be tested in later stage of infection. Therefore there were fewer censored observations in the group of children than in the adult group which may bias the statement regarding VC duration and VL. Also, as children are more likely to have asymptomatic courses of infection, they are more likely to be tested in later stages of infection, which can lead to underestimating time needed for viral clearance. We strove to account for this bias by meticulously reconstructing the probable date of infection, using comprehensive data on exposition, symptom onset of contact persons, and antibody test results.

Third, as the data collection for our study was set from 10/2020 to 6/2021, when the SARS-CoV-2 wild type and alpha VOC were dominant, it is unclear whether conclusions can be drawn about other variants.

Implications for policy and practice

The observation that it usually takes several weeks for children aged 1–6 years to eliminate SARS-CoV-2 from one's respiratory tract emphasizes that SARS-CoV-2 cases in daycare programs among enrolled children should be taken seriously, and isolation for infected children and quarantine of (non-immunized) close contacts need to be adhered to for an adequate span of time. If estimated from the first positive PCR test, reaching viral clearance took a median of 9 days, which may be an information that might be more useful for the real-life situation in daycare centers.

Our data also show, on the other hand, that persisting positive tests may not necessarily correspond to a child's infectiousness. In our study the vast majority of positive SARS-CoV-2 PCR test results beyond day 15 after the probable infection yielded a viral load well under the threshold of 10^6 RNA copies/ml. Viral load is recognized as a strong determinant of transmission risk (27) and according to our data, there are probably only few days when a child's dose of viral shedding is high enough to transmit the virus to others. These findings may add to other data which inform decisions on the length of isolation periods for children and adults. Other factors besides viral shedding (type of contact, respiratory volume, coughing, etc.) also play explicit roles in transmission, and viral shedding alone cannot be equated with transmission. In addition to epidemiological research findings on SARS-CoV-2 transmission in daycare-aged children, psychosocial and educational aspects should be considered for the selection of appropriate containment measures in the daycare setting.

Conclusion

On the whole, our study does neither fuel fears that children may be significant drivers of the COVID-19 pandemic, nor confirm theories that they hardly play a role in transmission dynamics. The viral load kinetics and transmission risks of young children are similar to those of adults, which supports to maintain containment measures in the daycare setting in order to provide protection to children and staff members.

Data availability statement

The datasets presented in this article are not readily available because the authors confirm that some access restrictions apply to the data underlying the findings. The data set cannot be made publicly available because informed consent from study participants did not cover public deposition of data. However, the minimal data set underlying the findings is archived in the "Health Monitoring" Research Data Centre at the Robert Koch Institute (RKI) and can be accessed by researchers on reasonable request. On-site access to the data set is possible at the Secure Data Center of the RKI's "Health Monitoring" Research Data Center. Requests to access the datasets should be directed to "Health Monitoring" Research Data Center, Robert Koch Institute, Berlin, Germany (*fdz@rki.de*).

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of the Berlin Medical Association. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JL, ASch, UB, WH and SJ designed the study. JW, HI, ASa, UK and BF were responsible for participant recruitment, data and specimen collection. AN, JM, LS performed laboratory testing and analysed lab results. ASR, JW, SD, ASch, TK, ASa, and GV carried out the analysis and were responsible for the accuracy of the data analysis. ASa, and JL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Case report: Histological findings of peri-appendicitis in three children with SARS-CoV-2 – related multisystem inflammatory syndrome: A mark for systemic inflammation?

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Background: Multisystem inflammatory syndrome in children (MIS-C) is a rare but serious condition that can potentially develop after SARS-CoV-2 infection in children. Gastrointestinal manifestation in MIS-C can mimic acute abdomen, potentially leading to unnecessary surgical treatment. Immune-mediated mechanisms seem to be a determining factor in its pathogenesis, and histological studies can help to shed light on this aspect. We describe three cases of children diagnosed with MIS-C that underwent appendectomy.
Methods: We retrospectively collected the clinical features and histological findings of three previously healthy children who underwent appendectomy for clinical suspicion of acute appendicitis but were later diagnosed with MIS-C.
Findings: The three children presented with prominent abdominal manifestations and fever leading to the suspicion of acute abdomen. Histological findings showed transmural and perivascular inflammation. Notably, $CD68^+$ macrophages were predominant in the child with milder abdominal symptoms without cardiac injury, while $CD3^+$ lymphocytes in the patient presented with more severe abdominal pain and cardiovascular involvement at admission.
Interpretation: Gastrointestinal symptoms of children with MIS-C improve after proper immunomodulatory therapy, conversely showing inadequate response to surgical appendectomy. Histological findings revealed different inflammatory cell infiltration that primarily involved perivisceral fat and vessels, and subsequently mucosal tissue, in contrast to other forms of acute appendicitis. Our findings suggest that this kind of peri-appendicitis in MIS-C could represent a focal sign of systemic inflammation, with different histological patterns compared to other forms of acute appendicitis.

KEYWORDS

MIS-C, COVID-19, inflammation, histology, appendicitis

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the pediatric population has a wide clinical spectrum, but it is frequently a mild disease and children usually recover within a few weeks (1).

Respiratory and gastrointestinal (GI) symptoms, such as abdominal pain, vomiting, and diarrhea are common findings in both adults and children during the acute stage of the SARS-CoV-2 infection (2–5).

Following the spread of the Coronavirus Disease (COVID)-19 pandemic, since May 2020, an increasing number of cases of a new condition sharing similarities with Kawasaki disease have been described. The World Health Organization named this newly identified condition as COVID-19-associated multisystem inflammatory syndrome in children (MIS-C) (6). MIS-C is a rare but serious systemic vasculitis, potentially developing four-to-six weeks after SARS-CoV-2 infection in children, characterized by clinical and laboratoristic signs of systemic inflammation and the involvement of two or more organs (7, 8), sometimes requiring intensive care unit (ICU) admission (8, 9). Since abdominal manifestations can be prominent and severe and may mimic acute abdomen, MIS-C can be misdiagnosed as a surgical emergency, potentially leading to unnecessary surgical treatment (8–12). A recent review of patients with MIS-C and severe GI symptoms showed that appendicitis was initially suspected in 5%–30% of patients (13).

The ileum and colon are the most frequently affected sites of the GI tract (2) in MIS-C and common echographic findings are ileitis, colitis, and lymphoadenitis (13), inflammation of the mesenteric adipose tissue, thickening of the terminal ileum and free abdominal effusion (14). Furthermore, MIS-C patients with prominent GI involvement usually exhibit lymphopenia, hypoalbuminemia, and increased D-dimer and fibrinogen levels with remarkably high C-reactive protein (CRP) at blood tests (2, 15). With these features, GI involvement in MIS-C can mimic other inflammatory bowel diseases (2), acute abdomen, and surgical emergencies (16–18) as well as other clinical conditions that can simulate acute appendicitis and thus be classified as pseudo-appendicitis (19).

The pathogenesis of the GI injury in MIS-C is far from being fully understood, although it seems to be mostly ascribable to immune-mediated mechanisms (14). Histological studies can help to shed light on this aspect, but to date, few cases describing the histological alterations of GI involvement in children with MIS-C have been reported in the literature (1, 14, 15). Indeed, when exploratory abdominal surgery was performed in such cases, histological studies revealed diffuse inflammation of the intestine and/or mesenteric lymphadenitis (14), without evidence of a viral cytopathic effect and without detectable viral particles (15).

In an attempt to partially fill this gap, we describe the clinical and histological features of three children affected by MIS-C with prominent abdominal involvement who underwent appendectomy before MIS-C diagnosis was made.

Materials and methods

We collected the surgical specimens of the appendices from children who underwent a surgical appendectomy in the suspicion of appendicitis and were later diagnosed with MIS-C from March to December 2021.

The diagnosis of MIS-C was done according to the definition of the World Health Organization (6), i.e., multisystemic involvement (at least two of the following symptoms: rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs, hypotension or shock, features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities, evidence of coagulopathy, acute GI symptoms), in the presence of elevated markers of inflammation (erythrocyte sedimentation rate, (ESR), C-reactive protein, (CRP), or procalcitonin) and fever that affected children and adolescents (0–19 years of age) exposed to SARS-CoV-2 (evidence of COVID-19 as demonstrated by positive RT-PCR test, antigen test or serology, or likely contact with patients with COVID-19) in the previous 2–6 weeks, without other obvious microbial cause of inflammation.

All GI manifestations (vomiting, diarrhea, abdominal pain, abdominal distension, paralytic ileus, pancreatitis, and pseudo-obstruction) were recorded. The presence of vomiting and diarrhea was documented based on standard definition if reported by caregivers and/or directly observed during the acute phase of the hospital stay. Abdominal pain was defined on children's self-reports of pain intensity using a Numerical Rating Scale (NRS) from 0 to 10 (14). Particularly, mild, moderate, and severe pain was defined by a score of at least 4, 6, or 8 out of 10, respectively. GI manifestations were suspected based on clinical presentation, imaging, and laboratory findings.

Blood tests at admission were collected and included: complete blood cell count (white blood cells, WBC; red blood cell count, RBC), CRP, procalcitonin, ferritin, interleukin-6 (IL-6), fibrinogen, D-dimer, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), albumin, sodium, creatine kinase (CK). Myocardial injury was evaluated by Troponin-I (cTnI) and B-type natriuretic peptide (BNP). In addition, a transthoracic echocardiogram (TTE) was performed in all patients during the acute and subacute phases and at a 6-month follow-up evaluation, to study left ventricular dimensions and function, presence of mitral regurgitation, and coronary dimensions, either as an absolute value (mm) and indexed by z-score.

The treatment of MIS-C consisted of intravenous immunoglobulins (IVIG) at 2 g/kg in a single infusion, methylprednisolone (MPD) at 2 mg/kg/day in case of shock and/or organ-threatening disease, aspirin at 3–5 mg/kg/day and enoxaparin sodium at 100 U/kg twice a day in case of elevation of D-Dimer more than 5 times above the normal values.

Surgical specimens of the appendix were sent to the Pathology Unit and routinely sampled. Two-µm-thick sections were cut from the formalin-fixed paraffin-embedded (FFPE) tissue blocks, for hematoxylin and eosin (H&E) stain and immunohistochemistry (IHC). IHC for CD68 (clone PG-M1) and CD3 (clone 2GV6) was automatically performed by means of automated immunostainer Benchmark® ultra (Ventana Medical Systems, Inc., Roche group, Tucson, AZ, United States), following the manufacturer's instruction. Negative controls are automatically performed at every IHC run by omitting the primary antibody.

Parental informed consent was obtained for all the children (protocol numbers: 98/2016/O/Sper and 178/2021/Sper/AOUBo).

TABLE 1 Laboratory tests, echocardiographic, and clinical features.

	Patient 1	Patient 2	Patient 3
Laboratory tests (normal values)			
WBC (×10^9/L) (4.8–12)	7.11	6.45	7
Neutrophils (%)	93.2	75.9	68
Lymphocytes (%)	5.1	18.4	16.4
Eosinophils (%)	0.10	0	0
MCV (fl) (76–91)	77	76	58
MCH (pg) (25–31.5)	26.5	26.4	18.8
Fibrinogen (mg/dl) (150–400)	403	445	–
D-dimer test (mg/L) (<0.55)	2.39	1.48	4.08
Creatinine (mg/dl)	0.32	0.30	0.41
Urea (mg/dl) (11–38)	15	21	25
Serum Sodium (mmol/L) (136–145)	130	134	133
Serum Potassium (mmol/L) (3.5–5.3)	4.5	4.2	4.1
Serum Albumin (g/L) (35–50)	31	26.3	38.7
Serum Total Protein (g/dl) (5.7–8)	7.7	7.5	6.2
Tpn I (ng/L) (<11.6)	9	50.9	2.4
BNP (pg/ml) (<100)	–	375	12
Creatine Kinase (UI/L) (<145)	42	31	45
IL-6 (pg/ml) (<6.4)	27.4	5.1	7.7
Ferritin (pg/ml) (11–306)	394	–	163
CRP (mg/dl) (<0.5)	16.81	19.25	18.66
Procalcitonin (ng/ml) (<0.5)	17.50	20.8	3.8
Echocardiography at admission			
Mitral regurgitation (yes/no)	No	Yes	No
Pericardial effusion (yes/no)	No	No	No
LVEF (%)	52	47	59
Clinical Data			
ICU admission (yes/no)	No	Yes	No
LOS (days)	10	8	7
Total days of fever	7	3	3
Time from the onset of fever to proper MIS-C treatment (days)	5	3	4

Legend: WBC, white blood cells; N, neutrophiles, L, lymphocytes, RBC, red blood cells; Hb, hemoglobin; PLT, platelets count; AST, aspartate-aminotransferase; ALT, alanine-aminotransferase; Tpn, Troponin I; BNP, brain natriuretic peptide; IL-6, Interleukin-6; CRP, C reactive protein; LVEF, left ventricular ejection fraction; ICU, intensive care unit; LOS, length of hospital stay.

Results

We collected data from 3 previously healthy Caucasian children (2 boys, aged 7–9 years), presenting to the emergency department (ED) with GI and systemic symptoms starting less than 5 days (median 3 days) before hospitalization. None of them was vaccinated against SARS-CoV-2. One patient (patient 1) presented with hypovolemic shock and one (patient 2) required admission to the ICU because of cardiac dysfunction occurring after the surgical procedure. Clinical and radiological features were all consistent with acute abdomen.

Laboratory tests, echocardiographic alterations, and clinical course of the patients are displayed in Table 1. All patients underwent surgical appendectomy before starting the proper MIS-C treatment.

The median hospital stay was 8 days.

Patient 1: A 7-year-old boy presented to the ED with a four-day history of vomiting, diarrhea, abdomen distension, and severe pain to the lower right abdomen (8/10 on NRS). Upon admission, he was pale and drowsy, febrile (40.5°C), tachycardic (135 bpm), and hypotensive (80/40 mmHg). His abdomen was diffusely tense and distended with a positive Blumberg sign. Two 10 ml/kg-boluses of isotonic crystalloid solution were given with subsequent improvement of vital signs. Blood tests revealed increased inflammatory markers, anemia, and thrombocytopenia. Abdominal ultrasound showed marked terminal ileitis with inflammation extending through the ascending colon, mesenteric fat stranding, and mesenteric lymphadenopathy in the right lower quadrant; the appendicular diameter was 6 mm. Echocardiography documented a mild decrease in left ventricular ejection function (LVEF) and dilation of the right coronary artery (z-score 2.3).

In the suspect of acute appendicitis, he underwent transumbilical laparoscopic-assisted appendectomy (TULAA). Endoscopic evaluation of the appendix did not show frank inflammatory signs. The main histopathological features revealed moderate-to-severe peri-appendiceal inflammation and neoangiogenesis. Particularly, the visceral wall and

perivisceral veins were infiltrated predominantly by $CD3^+$ lymphocytes, with a minor amount of $CD68^+$ macrophages (Figure 1).

After surgery, his clinical condition did not improve: he was prostrated and presented with high-grade fever, and diffuse abdominal pain persisted. Familiar history turned out to be positive for COVID-19 five weeks before, so the child was tested for SARS-CoV-2 serology, showing positive for previous infection. A diagnosis of MIS-C was thus made and the treatment with IVIG, MPD, acetylsalicylic acid, and enoxaparin sodium was started. After 48 h his clinical status and abdominal manifestations improved. At the 6-month follow-up, cardiac function and coronary dimensions had returned to normal.

Patient 2: A 9-year-old boy presented to the ED with a four-day course of fever (39.5°C) and asthenia, nausea, and moderate abdominal pain (6/10 on NRS). He presented with a tense and tender abdomen, particularly in the right lower quadrant, and positive Blumberg sign; moreover, he had signs of mild dehydration (dry mucous membranes and skin, fuzzy tongue, dull eyes), but his vital signs were normal (blood pressure 108/70 mmHg, heart rate 110 bpm, refill time <2 s, blood oxygen saturation levels 100% in room air). Blood tests showed increased markers of inflammation, anemia, thrombocytopenia, and slightly elevated cTnI. Abdominal ultrasound showed aperistaltic and dilated appendix (11 mm) with echogenic prominent perivisceral fat.

Intravenous rehydration with an isotonic solution was started and video-laparoscopic appendectomy was performed on the suspect of acute appendicitis. Histopathological findings showed mild transmural and perivascular inflammation. The inflammatory infiltrates were dominated by $CD68^+$ macrophages and $CD3^+$ lymphocytes in equal distribution (Figure 2).

After 48 h, his clinical conditions worsened with persistent high-grade fever, oxygen desaturation (85% in room air), and systemic shock (78/34 mmHg). Echocardiography documented mild left ventricular dysfunction (EF: 47%) and mitral insufficiency, while coronary arteries diameters were normal. He was, therefore, admitted to ICU requiring intensive monitoring and inotropic and respiratory support. Chest x-ray showed signs of pulmonary edema with bilateral pleural effusion and basal congestion. In the suspicion of an MIS-C, SARS-CoV-2 serology was performed, resulting positive for previous infection, despite negative familiar and personal history of COVID-19 and no vaccination. The proper therapy for MIS-C was started with subsequent improvement after 36 h.

He gradually recovered and was discharged after 8 days of hospitalization. At the 6-month follow-up, cardiac function and mitral regurgitation had returned to normal.

Patient 3: An 8-year-old girl was admitted to the ED for a high-grade fever (39.5 °C) lasting for 3 days, headache, and lower limbs muscular pain. Personal history was positive for an asymptomatic SARS-CoV-2 infection three weeks before the onset of abdominal pain. At admission, she was suffering from mild right lower abdominal pain and diffuse abdominal distension with positive Blumberg sign (5/10 on NRS), vomiting, and diarrhea; vital signs were normal (blood pressure 108/71 mmHg, heart rate 125 bpm, blood oxygen saturation 99% in room air). Abdominal ultrasound showed a mild thickening of the ileal wall to ascending colon and perivisceral fluid in the Douglas' pouch, without clear signs of

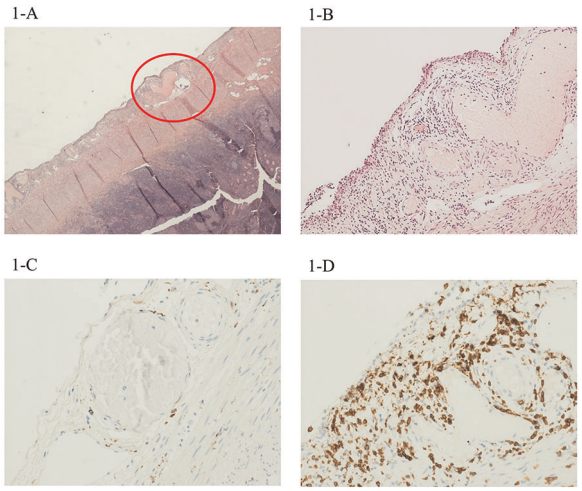

FIGURE 1
H&E image of the first case, with moderate-to-severe periappendiceal inflammation, involving the wall of perivisceral veins. This infiltrate showed relatively few $CD68^+$ macrophages (**C**) and several $CD3^+$ lymphocytes (**D**). Magnification 4× (**B**) and 20× (**A**).

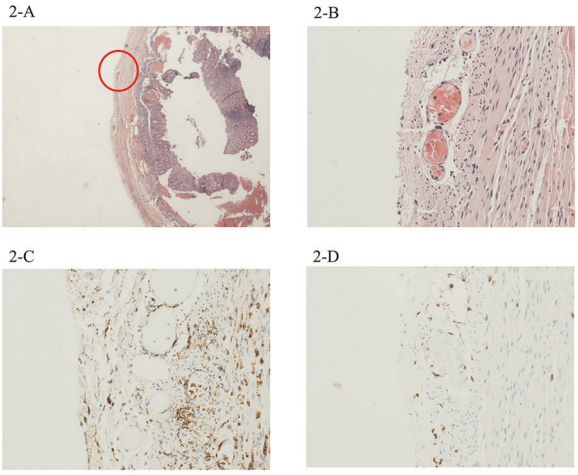

FIGURE 2
The second case showed mild inflammation, but with a perivascular involvement mostly by $CD68^+$ macrophages (**C**), with very few $CD3^+$ lymphocytes (**D**). Magnification 4× (**B**) and 20× (**A**).

acute appendicitis. Cardiac function and coronary arteries were normal.

She underwent to explorative laparoscopy procedure with complementary laparoscopic appendectomy. Histological findings revealed mild transmural and perivascular inflammation with an inflammatory infiltrate mostly represented by $CD68^+$ macrophages with very few $CD3^+$ lymphocytes (**Figure 3**).

In the suspicion of MIS-C, she started standard treatment with prompt improvement. She was discharged after 7 days.

Discussion

Children affected by MIS-C can experiment moderate-to-severe GI symptoms that appear to be associated with increased inflammatory markers, a more severe clinical course, and an increased risk of admission to the ICU (2). Furthermore, GI involvement in MIS-C can mimic acute abdomen, requiring differential diagnosis with surgical emergencies, particularly appendicitis, eventually leading to exploratory laparotomies (14). Nevertheless, laparoscopic or surgical appendectomy in children with MIS-C is not usually indicated, since it does not seem to improve the clinical course of the disease, and exposes these already critically ill patients to unnecessary anesthesiologic and surgical risks (13).

The histopathologic features in MIS-C described so far showed transmural and vascular inflammation of the affected GI tract. Particularly, transmural lymphocytic inflammation and focal acute enteritis involving mesentery and adjacent vessels of the ileum without appendicular involvement was described in a patient undergoing ileocolic resection: venous microthrombi originated in the subendothelial space widely affected mucosa, submucosa, and subserosa of the terminal ileum (15).

In our patients, the main histological findings were inflammatory infiltrates involving the wall of several arteries and veins in the peri-appendiceal fat, with associated neoangiogenesis. IHC revealed that the composition of this perivascular transmural infiltrate significantly differed among patients according to clinical severity. Particularly, perivascular inflammation was characterized by a significant proportion of $CD68^+$ macrophages in the child with milder abdominal symptoms without cardiac injury (case 3), while $CD3^+$ lymphocytes were predominant in the patient presenting with more severe abdominal pain and cardiovascular involvement at admission with shock, ventricular dysfunction, and coronary dilation.

Notably, in the usual presentation of acute appendicitis, a predominantly neutrophilic granulocyte-rich inflammation typically starts in the mucosa, eventually spreading through the wall up to the serosa. Indeed, perivisceral inflammation is described in more than half of cases of acute pediatric appendicitis, mostly with moderate-to-severe inflammation (20). In our cases, inflammation of the serosa was present even if mucosal inflammation was none-to-mild, in line with previous observations on the extra-pulmonary manifestations of SARS-CoV-2 disease (21, 22).

It is difficult to assess whether this acute peri-appendicitis is just a comorbidity in children with MIS-C or if it is a clinical and histopathological part of the systemic inflammatory process, potentially involving multiple organs and systems. Albeit local, perivascular inflammation in the appendix of children with MIS-C likely represents the focal sign of a diffuse disease and a systemic inflammation involving the entire GI tract and potentially other sites. Strong support for the systemic nature of MIS-C to intestinal and appendicular inflammation comes from the response to proper MIS-C treatment, since all patients rapidly improved after immunomodulatory therapy, rather than after surgical procedure. It is still unclear whether the inflammatory findings are determined by direct viral-induced cellular damage or if they are the consequence of a systemic inflammatory process affecting the GI tract (15, 23).

Of note, neither viral particles nor viral cytopathic effect was detected in any of the histopathological reports published so far (15, 21), highly suggesting an immune-mediated mechanism rather than a direct viral injury. Furthermore, the pathological findings from the three cases show that inflammatory infiltrate primarily affects the perivisceral fat and vessels, in contrast with other forms of acute appendicitis in which inflammation extends from the mucosal surface to the perivisceral fat.

Interestingly, "pseudo-appendicitis" can be the signature of other diseases, such as inflammatory bowel diseases (IBD), mainly Crohn's disease (CD), *Bartonella*-related ileitis, *Yersinia enterocolitica* infection, and Kawasaki disease (KD) with intestinal involvement, with whom differential diagnosis must be considered (24). *Bartonella henselae*, for instance, causes infectious diseases that can present as acute ileitis and systemic symptoms such as fever and lymphoadenitis (25, 26). *Y. enterocolitica* (27) and *Yersinia pseudo-tuberculosis* (19) infections share similar clinical features, blood test alterations, and abdominal echographic findings with acute appendicitis. Histological findings in *Y. enterocolitica* infection are aspecific, documenting a peri-appendicitis characterized by mixed acute and chronic inflammation infiltrates, focal neutrophilic cryptis, and epithelial cell granulomas composed by small T-lymphocytes, plasma monocytes, and hystiocytes (28). However, diagnosis of *Y. enterocolitica* infection is made by isolation of the germ from biological samples (27).

On the other hand, non-infectious systemic diseases, such as IBD and KD can also mimic acute appendicitis, with peculiar histological features that differ from MIS-C pathological findings.

When the appendix is concerned, transmural inflammation with fibrous thickening of the wall, non-caseating granulomas,

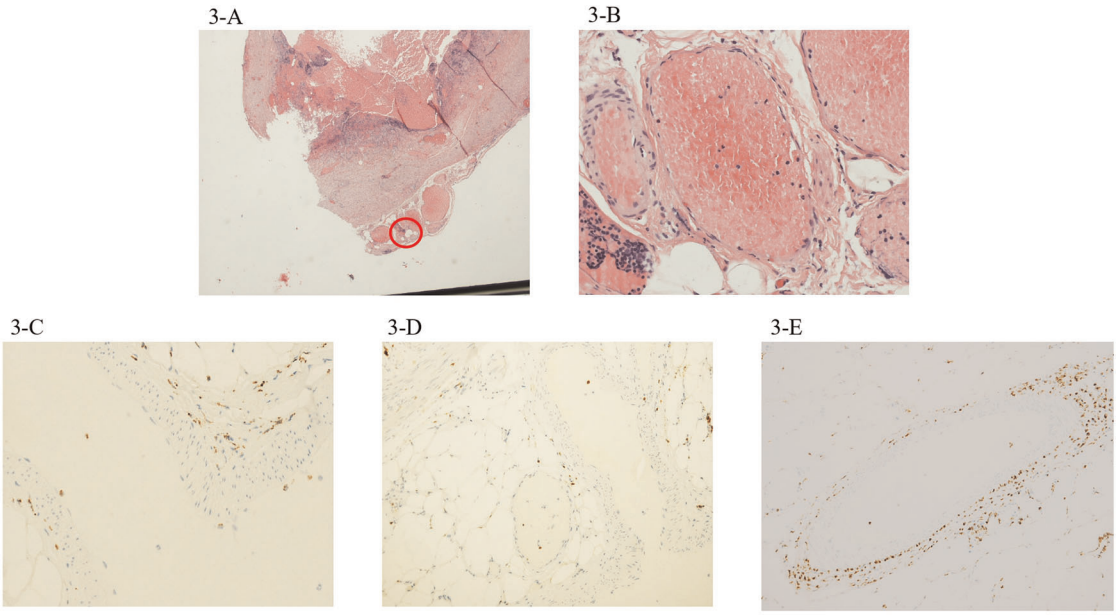

FIGURE 3
The third case showed mild inflammation, with a scarcity of both perivascular CD68$^+$ macrophages (C) and CD3$^+$ lymphocytes (D). Magnification 4× (B) and 20× (A). A detail of the CD68$^+$ macrophagic infiltrate around and within a large periappendiceal vein in case 2 (E).

muscular hypertrophy, and crypt abscesses are documented (29). Typical extra-intestinal manifestations such as arthralgia, uveitis, and arthritis (30), and Anti-*Saccharomyces cerevisiae* antibodies can lead to a diagnosis of CD.

Appendicitis in KD is very rare, as opposed to frequent abdominal symptoms (31). A large number of IgA plasma cells, likely due to the stimulation of the systemic IgA immune system, were found in the GI tract in patients with KD, but also in non-KD controls (32). In the 8 cases of KD with appendicitis published so far, histological findings showed found focal inflammation with neutrophilic and eosinophilic infiltration in one patient and transmural inflammation and arteritis in another one (33). In case of persistent postoperative fever, especially if other systemic signs appear, the diagnosis should be reconsidered, with the awareness that KD tends to affect younger patients than MIS-C (34).

Conclusion

Our findings support the hypothesis that peri-appendicitis in children with MIS-C might represent a manifestation of multisystemic inflammation. Notably, the perivascular transmural infiltrate differed among patients with a significant proportion of CD3$^+$ lymphocytes in presence of more severe abdominal pain and cardiovascular involvement at admission, while CD68$^+$ macrophages were predominant when intestinal symptoms were milder without cardiac injury. The cell infiltration patterns could suggest that different cells are involved in the inflammatory response and may indicate different stages or expressions of the same pathological process. Pediatricians and pediatric surgeons must be aware of this clinical presentation of MIS-C in order to properly treat the patients and avoid unnecessary surgery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

MF, FV and FG had a prominent role in the conceptualization, data curation, investigation, and methodology of the manuscript. They wrote, reviewed, and edited the manuscript. AD, AR, ML and ML provided substantial contribution to the data curation, validation, supervision, and methodology of the manuscript. They

collaborated to write, review, and edit of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Development of restrictive eating disorders in children and adolescents with long-COVID-associated smell and taste dysfunction

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Background: Absent or abnormal senses of smell and taste have been frequently reported during both acute and long COVID in adult patients. In contrast, pediatric patients who test positive for SARS-CoV-2 are often asymptomatic and the loss of smell and/or taste has been infrequently reported. After observing several young patients with COVID-associated anosmia and ageusia at our clinic, we decided to investigate the incidence of subsequent eating disorders in these patients and in SARS-CoV-2 positive patients who did not experience anosmia and ageusia during the same period.
Material and methods: A single-site retrospective cohort study of 84 pediatric patients with suspected long COVID who were treated in the Pediatric Infectious Diseases Outpatient Clinic at the University Hospital Essen were evaluated for persistent symptoms of COVID-19. Smell and taste dysfunction as well as eating behaviors were among the signs and symptoms analyzed in this study.
Results: 24 out of 84 children and adolescents described smell and taste dysfunction after confirmed or suspected SARS-CoV-2 infections. A large number of these patients (6 out of 24) demonstrated increased fixation on their eating behavior post-COVID and over time these patients developed anorexia nervosa.

Abbreviations
AWMF, German national guidelines; BAU, Binding antibody units; BMI, Body mass index; CI, Confidence interval; ECG, Electrocardiogram; ECHO, Electrocardiography; ED, Eating disorder; EEG, Electroencephalogram; ENT, Ear Nose and Throat Specialist; ICD-11, International Classification of Disease 11; kg, Kilogram; LC, Long COVID; LCI, Lung Clearance Index; m^2, Square meter; MRI, Magnetic Resonance Imaging; N, Number; NCV, Nerve Conduction Velocity; Perc, Percentile; PIMS, Pediatric-Inflammatory-Multisystem-Syndrome; SAT, Smell and taste dysfunction; SCOFF, Anorexia questionnaire ("Sick, Control, One, Fat, Food").

Discussion/Conclusion: In this study we saw a possible association of long-lasting post-COVID smell and taste dysfunction with subsequent development of eating disorders. This observation is worrisome and merits further investigation by healthcare providers at multiple clinical sites.

KEYWORDS

pediatric long COVID, anosmia, ageusia, eating disorder, anorexia

Introduction

Since the first patient with SARS-CoV-2 pneumonia was identified in Wuhan China in December 2019, significant morbidity and mortality associated with both the acute and long-term effects of COVID in adults has been reported worldwide. However, in children and adolescents, the majority of SARS-CoV-2-PCR-positive patients remain asymptomatic. Only a few pediatric patients present with severe acute COVID-19 (1). Apart from the acute disease, different courses of illnesses associated with previous SARS-CoV-2 infection have been reported such as Pediatric-Inflammatory-Multisystem-Syndrome temporally associated with SARS-CoV-2 (PIMS) (2–5) or long COVID and "Post-COVID-Syndrome" (6, 7).

While the two latter diagnoses are clearly defined for adults (6, 7), no clear case definition for pediatric patients exists, although children and adolescents display similar symptoms such as fatigue, muscle pain, headaches, concentration difficulty, and/or olfactory and gustatory dysfunction (8, 9). In adults, symptoms of long COVID, especially persistent olfactory dysfunction, are associated with a lower quality of life and a negative impact on mental health (10, 11). The prevalence of childhood long COVID remains unclear, as very few studies exist and among them even fewer that include a control group (10).

Moreover, the COVID pandemic has caused an increase in mental stress in children, adolescents, and their families worldwide. A rise in depression and anxiety disorders has been reported, as reflected by increased admissions due to severe eating disorders and suicide attempts (5,12–15).

During the treatment course of children and adolescents with suspected long COVID, we observed a possible association between changes in smell and taste and restrictive eating behavior. Recently, other studies have also described significantly disturbed eating habits in children with changes in smell and taste due to Long COVID (16). Several reports have revealed an increased incidence of anorexia nervosa after the onset of COVID-19 and associated measures to control the epidemic (17, 18). Hence, we retrospectively investigated smell and taste dysfunction (SAT) in our cohort to determine whether such a dysfunction represented a symptom of long COVID and whether SAT led to the development of anorexia nervosa or another eating disorder-associated disease.

Material and methods

Study design

Our investigation consisted of a single-site, retrospective cohort study of patients treated in the Pediatric Infectious Diseases Outpatient Clinic at the Department of Pediatrics I, University Hospital Essen. Data were collected from existing patient records.

Ethics approval

This study was approved by the Ethics Committee of the Medical Faculty of the University Duisburg-Essen (22-10581-BO) and conducted in accordance with the latest version of the Declaration of Helsinki. As the study presents a retrospective analysis with anonymous data, the ethics committee waived the need for informed consent.

Eligibility criteria

Included were all patients (age 0–18 years, $n = 84$) treated in the Pediatric Infectious Diseases Outpatient Clinic at the Department of Pediatrics I, University Hospital Essen from April 2021 to April 2022 with a diagnosis of suspected long COVID. Twenty-four patients, who met the criteria of long COVID according to the German Association of the Scientific Medical Societies (AWMF) and NICE guidelines (6, 7) and reported changes in their olfactory and/or gustatory senses, were further evaluated. These patients were divided into two groups according to the presence ("LC+SAT+ED"-group) or absence ("LC+SAT"-group) of restrictive eating behavior and anorexia nervosa as defined by the International Classification of Disease ICD-11 (19). Patients without changes in their eating behavior and no diagnosed eating disorder comprised the "LC+SAT"-group (see Diagram 1).

Exclusion criteria

In cases where the diagnosis of long COVID could not be confirmed ($n = 25$), SAT was not reported ($n = 30$), or the

long COVID diagnosis was rejected due to insufficient data (n = 5), patients were excluded.

Diagnostic workup

A detailed medical history, blood tests (see **Supplementary material**), physical examination, oxygen saturation, blood pressure, and a consultation with an Ear Nose and Throat (ENT) specialist were obtained from all patients. Body mass indices (BMI; kg/m^2) were assessed using BMI-centiles adapted to age, sex, and race (20). All further consultations with other medical specialists were conducted based on clinical presentation. Electroencephalogram (EEG), electrocardiogram (ECG), electrocardiography (ECHO), ultrasound of the abdomen and thyroid, chest-x-rays, lung function with lung clearance index (LCI) measurement, cranial or spinal magnetic resonance imaging (MRI), and Nerve Conduction Velocity (NCV) were performed if necessary. We recommended a consultation with a child and adolescent psychiatrist for all patients with newly developed restrictive eating behavior to rule out or confirm the diagnosis of a possible eating disorder and initiate treatment. Demographic variables, preexisting diagnoses, and newly detected underlying diseases as well as other known long COVID-associated symptoms (fatigue, headaches, etc.) were documented. Patients were systematically queried as to symptoms associated with long COVID and restrictive eating behavior and psychological or other somatic symptoms were included in the evaluation. Amenorrhea was defined by three or more missing menstrual cycles; menstruation was defined as irregular if a time shift of more than 30% had occurred for three months after at least three months of regular menstrual cycles. The subjective change in recalled symptoms over time was monitored. An improvement was defined by less reported intensity, quantity of symptoms, and/or alleviation of restrictions in daily routines, and numbers of symptoms or less restrictions in daily routines caused by long COVID symptoms.

Statistics

Statistical analysis was performed with SPSS 27 Statistic Software (IBM; Armonk, NY). Continuous variables were presented as median. The confidence interval (CI) was set at 95%. Mann-Whitney-U-test for continuous variables or Chi-Square test for categorical variables were used to test for differences between cases and control groups. The level of significance was set at $p = 0.05$. Percentiles for height, weight, and BMI were used according to ICD-11, 6B80 Anorexia Nervosa.

Results

Twenty-four (28.6%) of the 84 patients evaluated in this study met both the criteria of long COVID according to the AWMF Guidelines (see **Table 1**) and had SAT. These symptoms were evaluated by systematic anamnesis and

TABLE 1 Definitions.

Definition	
AWMF Long COVID	–Persisting symptoms after acute COVID-19 or its treatment –Symptoms leading to a new health impairment –New symptoms that appeared after the end of the acute phase as a result of COVID-19 disease –Worsening of a pre-existing underlying condition (6)
NICE Long COVID	–Ongoing symptomatic signs and symptoms that developed during COVID-19 and have no other explanation from 4 weeks up to 12 weeks after the acute infection –>12 weeks = post-COVID-19 syndrome. It usually presents with clusters of symptoms, often overlapping, which can fluctuate and change over time and can affect any system in the body. (7)
Definition used for changes in olfactory senses	–Decreased taste and/or smell (Hyposmia/Hypogeusia) –Loss of taste and/or smell (Anosmia/Ageusia) –Unpleasant smell and/or taste, commonly a rancid odor/flavor
ICD-11 Definition Anorexia nervosa	–Significantly low body weight for the individual's height, age, developmental stage, or weight history (body mass index [BMI)] < 18.5 kg/m^2 in adults and under 5th percentile in children and adolescents; rapid weight loss [e.g., > than 20% of total body weight within 6 months]) –Low body weight not better accounted for by another medical condition or the unavailability of food –Persistent pattern of restrictive eating or other behavior aimed at establishing or maintaining abnormally low body weight, typically associated with extreme fear of weight gain –Excessive preoccupation with body weight or image (15)

excluded anatomic reasons (Table 2 and Diagram 1 in Supplementary material). Six patients (3 male and 3 female) showed restrictive eating behavior and 5 of them met the ICD-11 guidelines for anorexia nervosa (25% of the children with SAT and 8.6% of the entire group with suspected long COVID) (see Diagram 1). None of the long COVID patients without SAT showed differences in their eating behavior or developed anorexia nervosa.

The 24 patients were ages 6–17 years. SARS-CoV-2 infection had been confirmed by PCR tests in all patients. None of the 24 patients with SAT had been hospitalized due to COVID-19. None of them suffered from gastrointestinal symptoms during acute or long COVID. Long COVID symptoms without an association to eating disorders included: decreased general condition (87.5%), "brain fog"/ concentration difficulty (37.5%), hyperesthesia (50.0%), and headaches (75.0%). There were no significant differences between concentrations of SARS-CoV-2 antibodies in the "LC + SAT + ED"-group and "LC + SAT"-group (median: 125.6 BAU/ml [binding antibody units/mL] vs. 450 BAU/ml; standard deviation 870.8). Additionally, there were no significant differences in SARS-CoV-2 viral variants between the two groups (see Table 3).

Five of the 6 patients with restrictive eating behavior reported a reduced appetite. This symptom, as well as hair loss or changes in menstrual cycle were significantly more common in the "LC+SAT+ED"-group than in the "LC+SAT"-group (hair loss $p = 0.001$, menstrual cycle $p = 0.042$, Chi-quadrant). In addition, 5 patients reported symptoms of depression, stress, and no longer enjoying food. Noticeably all patients felt misunderstood by family, friends, and professionals. Depressive symptoms and anxiety were significantly more common in the "LC+SAT+ED"-group than in the "LC+SAT"-group ($p = 0{,}005$, Mann-Whitney-*U*-test, $z = -2{,}835$) (see Table 4); one of the "LC+SAT"-group patients had been diagnosed with depression and an eating disorder before COVID infection, which did not worsen during the course of disease.

None of the "LC+SAT+ED"-group or "LC+SAT"-group patients followed a special diet (e.g., vegetarian, vegan, or gluten-free). The 6 patients of the "LC+SAT+ED"-group reported restrictive eating behavior and lost between 4 and 6.5 kilograms of body weight (see Tables 3, 5). The period from the beginning of long COVID symptoms to the maximum loss of weight was between 3 and 5 months. Four of the patients reported a distorted body image and two of them had to weigh themselves frequently. No compensatory mechanisms were reported. Associated symptoms like decreased general condition, amenorrhea, constipation, and malnutrition also existed (see Table 4). None of the "LC+SAT"-group patients lost weight (see Table 3). Weight loss and BMI reduction during long COVID were significantly higher in the the 6 patients of the "LC+SAT+ED"-group ($p < 0.001$, $p = 0.009$, chi-quadrant, Table 4) than in patients of the "LC+SAT"-group.

Treatment recommendations included training of the olfactory nerve (twice a day smelling of 4 different essential oils, each for a period of 10 s), keeping a diary of symptoms, weight check-ups by a pediatrician, consultations with a child and adolescent psychiatrist, and provision of emergency contacts to both patients and their families (Table 2). These recommendations were made with the intention of symptom

TABLE 2 Diagnostics and therapy.

	LC + SAT + ED ($n = 6$)		LC + SAT ($n = 18$)	
Diagnostic Procedures	Pathological: Echocardiography: 1 MRI: 1 (cranial, post mastoiditis)	Normal: lung function: 6 ECG: 4 Echocardiography: 2 Chest x-ray: 3 MRI: 3 planned NCV: 1 (1 planned) EEG: 5 ultrasound:	Pathological: lung function: 4 ECG: 1 Echocardiography: 1 EEG: 2 ultrasound: 1 NCV: 1 chest x-ray: 1	Normal: lung function: 12 ECG: 13 Echocardiography: 14 chest x-ray: 6 MRI: 3 NCV: 2 EEG: 4 ultrasound: 5
Consultants	Pathological: Child and adolescence psychologist: 5	Normal: NCV: 5 Neuropediatric: 4 Ophthalmology: 3	Pathological: Pneumology: 3 Neuropediatric: 1 Ophthalmology: 1	Normal: Neuropediatric: 4 Ophthalmology: 1 Pneumology: 1 Cardiology: 1 Gastroenterology: 1
Therapy	Smell training: 5 Psychological consultation: 4 Mother child rehabilitation: 1 Symptom diary: 2		Smell training: 16 Psychological consultation: 2 Physiotherapy: 2 Mother child rehabilitation: 1 Symptom diary: 2 Lifestyle changes: 2	

TABLE 3 Patient characteristics and COVID-19 associated symptoms.

	LC+SAT+ED (*n* = 6)	*LC+SAT* (*n* = 18)
Age (years)	8–14 (Median 11.5)	6–17 (Median 14)
Sex	3 female, 3 male	13 female, 5 male
Numbers of MD consultations since the first signs of long COVID before presentation at our Pediatric Infectious Diseases Outpatient Clinic	1–5 (Median 2.5) Fever: 4 (66.7%) Flu-like symptoms: 3 (50.0%) Chest pain: 2 (33.3%) Headache: 5 (83.3%)	1–5 (Median 3) Fever: 4 (22.2%) Flu-like symptoms: 13 (72.2%) Chest pain: 1 (5.6%) Headache: 11 (61.1%)
Initial symptoms during Covid 19	Smell and taste dysfunction: 1 (16.7%) Pain in Extremities: 1 (16.7%) Vertigo: 2 (33.3%) Diarrhea: 0 (0.0%) Unknown: 1	Smell and taste dysfunction: 1 (5.6%) Pain in Extremities: 1 (5.6%) Vertigo: 9 (50.0%) Diarrhea: 1 (5.6%) Unknown: 10
Subtype of SARS-CoV2 (PCR)	Delta/Indian B.1.617.2: 4 Alpha/British B.1.1.7: 0 Omicron B.1.1.529: 2	Delta/Indian B.1.617.2: 5 Alpha/British B.1.1.7: 1 Omicron B.1.1.529: 2
SARS-CoV2-antibodies (BAU/ml)	2020–0 (Median: 126)	>2090–0 (Median: 322)
Max. Loss of weight after COVID-19 (kilogram)	4–6.5 (Median: 5) 95%-CI: 4.33–6.17 Before long COVID: 18.1–23.1 95%-CI: 17.7–22.1 Actual BMI:	1 (No median, only 1 patient) 95%-CI: 0.0–0.17 Before long COVID: 15.5–25.7 95%-CI: 18.1–21.8 Actual BMI:
Actual body mass index (and before long COVID) (kg/m^2)	14.8-18.4 95%-CI: 15.4–18.3 BMI reduction: 95%-CI: 1.77-5.58	15.5-25.7 95%-CI: 18.2–22.1 BMI reduction: 95%-CI: 0.0–0.18
Days until the symptoms improved	0–84	0–120 days

management. In 5 of the 6 patients of the "LC+SAT+ED"-group, the diagnosis of anorexia nervosa was confirmed during further examinations by a child and adolescent psychiatrist or a psychologist. Three of the "LC+SAT+ED"-group patients reported fewer symptoms after 32 to 84 days. One of them reported decreased symptoms following SARS-CoV-2-vaccination (Pfizer/BioNTech). Three patients attributed their improvementto psychotherapy.

Discussion

Here, we describe a group of 24 pediatric patients ages 6–17 years with long COVID or Post-COVID Syndrome and concurrent SAT. A clinically important proportion of patients developed a restrictive eating behavior and secondary eating disorder as defined by ICD-11. SAT is a widely recognized symptom of acute SARS-CoV-2 infection. In cases of COVID-19 persistence for more than 4 weeks, the diagnosis of long COVID was confirmed; when persistence continued beyond 12 weeks, Post-COVID Syndrome was confirmed. A relevant impact of long COVID on the mental health in affected adults has already been documented (10). However, to our knowledge, the association of SAT dysfunction and eating disorders in childhood has not been reported to date.

Even if the prevalence of long COVID in children remains unclear (10), some studies report its ubiquity in children with symptoms like persistent fatigue (25.2%), cognitive sequelae such as irritability (24.3%) and mood changes (23.3%), headaches (16.9%), rhinorrhea (16.1%), coughing (14.4%), and anosmia/dysgeusia (12.3%) (8, 9). Patients included in this study presented with these symptoms.

Within the study population all patients with a secondary eating disorder showed somatic symptoms (reduced fitness, constipation, malnutrition and amenorrhea) and were ages 8–14 years. Normally, the incidence of eating disorders is highest for girls ages 15–19 years and for boys ages 10–14 years (21). In all 6 patients in this study, the focus on food and restrictive eating occurred after quality changes in smell and taste. Increased attention to eating behavior like obsession with the daily meal plan is a risk factor for the development of eating disorders and may have promoted their development (22). In adults, persistent olfactory dysfunction has been associated with a lower quality of life and impaired mental health (10).

TABLE 4 Long COVID symptoms and eating behavior.

	LC+SAT+ED ($n = 6$)	*LC+SAT* ($n = 18$)
Long-lasting GI symptoms	Changes in quality of taste and smell: 6 (100.0%) Reduced smell and taste: 3 (50.0%) Reduced appetite: 5 (83.3%) Abdominal pain: 0 Constipation: 2 (33.3%)	Changes in quality of taste and smell: 18 (100.0%) Reduced smelling and tasting: 9 (50.0%) Reduced appetite: 2 (11.1%) Abdominal pain: 3 (16.7%) Constipation: 4 (22.2%)
Somatic symptoms of eating disorder	Irregular menstrual cycle: 3 (100.0% of females) 95%-CI: 0.0-1.07 Hair loss: 3 (50.0%), 95%-CI: 0.00–1.07 Sleep problems: 3 (50.0%) Fatigue: 3 (50.0%) Less condition: 5 (83.3%) Headache: 4 (66.7%) Dyspnea: 1 (16.7%)	Irregular menstrual cycle: 2 (15.4% of females) 95%-CI: 0.0-0.27 Hair loss: 2 (11.1%), 95%-CI: 0.0-0.27 Sleep problems: 6 (33.3%) Fatigue: 6 (33.3%) Less condition: 16 (88.9%) Headache: 14 (77.8%) Dyspnea: 6 (33.3%)
Other long-lasting symptoms	Paresthesia: 2 (33.3%) Palpitation: 3 (50.0%) Concentration problems: 4 (66.7%) Chest pain: 1 (16.7%) Vertigo: 1 (16.7%)	Paresthesia: 10 (55.6%) Palpitation: 4 (22.2%) Concentration problems: 8 (44.4%) Chest pain: 1 (5.6%) Vertigo: 4 (22.2%)
Psychological symptoms	Self-reported depression symptoms: 5 (83.3%) Self-reported anxiety symptoms: 4 (66.7%) 95%-CI: 0.40–1.26	Self-reported depression symptoms: 5 (27.8%) Self-reported anxiety symptoms: 5 (27.8%) 95%-CI: 0.05–0.51
Restrictive eating behavior	Restrictive eating behavior: 6 95%-CI: 1.0–1.0	0 95%-CI: 0.0–0.0
Permanent weight control	2 (33.3%)	0
Body Scheme	Distorted body image: 4 (66.7%) Body checking: 2 (33.3%)	Distorted body image: 1 (5.6%)
Suicidal ideation	Suicidal thoughts: 1 (16.7%)	None

Table 5 Signs of eating disorder within the six described *LC+SAT+ED*

Signs of anorexia nervosa ICD 11	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**
	BMI before LC 21.3	BMI before LC 18.2	BMI before LC 23.1	BMI before LC 20.5	BMI before LC 18.2	BMI before LC 18.1
Body mass index (BMI) < 18.5 kg/m^2; < 5th percentile in children and adolescents; rapid weight loss (e.g., > than 20% of total body weight within 6 months)	lowest during LC: BMI 16.3 7. Percentile in 3 months 14% decrease	lowest during LC: BMI 16.2 8. Percentile in 3 months 14% decrease	lowest during LC: BMI 16.5 11. Percentile in 3/4 months 21% decrease	lowest during LC: BMI 17.4 14. Percentile in 3 months 21% decrease	lowest during LC: BMI 16.1 36. Percentile in 4 months 16% decrease	lowest during LC: BMI 14.8 5. Percentile in 5/6 months 19% decrease
Low body weight is not better accounted for by another medical condition or the unavailability of food.	No other explanations	No other explanations	No other explanations	No other explanations	No other explanations	No other explanations
A persistent pattern of restrictive eating or other behavior aimed at establishing or maintaining abnormally low body weight, typically associated with extreme fear of weight gain.	Yes	Yes	Yes	Yes	Yes	Yes
Excessive preoccupation with body weight or image.	Yes	Yes	Yes	Yes	Yes	Yes
Somatic effects of the eating disorder						
- Hair loss			X	X		X
- Changes in the menstrual cycle	X		X	X		X
- Less energy	X	X	X	X	X	

Female patients are usually more prone to develop an eating disorder than males. Only 5%–10% of patients who were reported with anorexia and bulimia nervosa during the COVID pandemic were male (23–26). Our results showed an equal distribution of both sexes who developed an eating disorder due to restrictive eating behavior. This implies that long COVID may affect male and female adolescents alike and override the usual gender differences in eating disorders, suggesting that SAT represents a clinically important individual risk factor for a new eating disorder.

Well documented research has proven that patients with eating disorders, especially with anorexia nervosa, are at risk of many somatic and psychiatric comorbidities and higher mortality (15, 27–30). Our study results show a positive correlation between restrictive eating behavior and symptoms of depression. These facts and the significant loss of weight in our patients emphasizes the importance of assessing both female and male pediatric patients for possible eating disorders after COVID-19. Physicians, caregivers, and patients should be able to recognize symptoms of an eating disorder, especially in all cases of SAT. Follow-up departments with multidisciplinary teams are needed for children and adolescents who experience signs of long COVID. In the case of SAT, screening methods like the SCOFF Questionnaire to detect eating disorders in children and adolescents at an early stage could be helpful (31).

There are several limitations to our study. As there is no acknowledged definition for long COVID in pediatric patients, we used the definition for adults which might not be suitable for pediatric patients in every sense. In addition, smell and taste dysfunctions are subjective symptoms. Only 5 patients were diagnosed by a mental health specialist prior to our study because of very limited resources due to higher demand for psychological and psychiatric services for children during the pandemic. Therefore, not all of our patients had the opportunity to get standard psychological evaluations during their visit in our outpatient clinic. Due to the different home addresses of patients included in this study and different geographic locations of their respective healthcare providers, the corresponding psychiatrist or psychologist was not always the same person. Furthermore, as long COVID is a relatively rare and new diagnosis, in this single-site retrospective cohort study we only report on a small patient group, which may result in statistical biases.

Conclusion

Pediatric patients with the diagnosis of long COVID and changes in smell and taste seem to be at risk of developing an eating disorder. Pediatricians should be aware of red flags while obtaining a medical history and performing a physical examination of these patients. As stated on many occasions, the mental health of children and young adults has suffered during the pandemic of SARS-CoV-2 (13). The possibility of consultation with a Child and Adolescent Psychiatrist or a Child Psychologist and access to necessary psychotherapies are urgently needed (32). Screening methods to detect secondary eating disorders in patients suffering from long COVID could be a helpful to pediatricians and child psychiatrists. Collaboration with child and adolescent psychiatrists as well as teachers, sports coaches, social workers, and families is even more necessary during the COVID-19 pandemic than in non-COVID-19 times. More research with larger patient groups is needed to support the hypothesis that changes in taste and smell due to long COVID are a risk for developing anorexia nervosa in pediatric patients. We hope to test this in a multicenter prospective study in the near future.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty of the University Duisburg-Essen (22-10581-BO). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

Study conception and design: SG, CDS, MB, JH; Acquisition of data: SG, MB; Analysis and interpretation of data: SG, CDS, MB, JH, NB; Drafting and revising the manuscript: SG, CDS, MB, BD, JH, NB, UF. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.1022669/full#supplementary-material.

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Multisystem inflammatory syndrome in children (MIS-C) and "Near MIS-C": A continuum?

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Introduction: Reports of multisystem inflammatory syndrome in children (MIS-C), following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, have been increasing worldwide, with an incidence varying significantly across studies based on the definition used for the diagnosis. At our tertiary medical center in Lebanon, we encountered several cases that presented a diagnostic challenge because they mimicked MIS-C but did not meet the US Centers for Disease Control and Prevention (CDC) definition. We decided to review these cases and describe their features in comparison with cases that met the CDC criteria of MIS-C and those that had an alternative diagnosis.

Methods: This is a retrospective chart review of subjects aged <19 years old admitted to the American University of Beirut Medical Center (AUBMC) between March 1, 2020, and May 31, 2021, with suspected or confirmed MIS-C, following documented COVID-19 infection, with sufficient or insufficient criteria for diagnosis. Subjects were classified into 3 groups: "MIS-C", "Near MIS-C" and "Alternative Diagnosis".

Results: A total number of 29 subjects were included in our cohort. Fever was present in all subjects. In the MIS-C group, evidence for cardiovascular system involvement was the most common feature followed by the mucocutaneous and gastrointestinal systems. In the "Near MIS-C" and "Alternative Diagnosis" group, gastrointestinal symptoms were the most common with only one patient with cardiac abnormalities and none with coagulopathy. Subjects with typical MIS-C presentation had higher inflammatory markers when compared to subjects in the other groups. Almost all the subjects had positive IgG for SARS-CoV-2. Of the 29 subjects, the Royal College of Paediatrics and Child Health (RCPCH) case definition would have identified all suspected cases without an alternative diagnosis as MIS-C, whereas the World Health Organization (WHO) and the CDC definitions would have excluded 6 and 10 subjects, respectively.

Conclusion: MIS-C presents a diagnostic challenge due to the nonspecific symptoms, lack of pathognomonic findings, and potentially fatal complications. More research is needed to fully understand its pathogenesis, clinical presentation spectrum, and diagnostic criteria. Based on our experience, we favor the hypothesis that MIS-C has a continuum of severity that necessitates revisiting and unifying the current definitions.

KEYWORDS

fever, COVID-19, SARS-CoV-2, multisystem inflammatory syndrome in children (MIS-C), Lebanon, children, adolescents

Introduction

A novel coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was recognized in December 2019 in China following the emergence of unexplained severe lower respiratory infections in clusters of patients (1, 2). The World Health Organization (WHO) has declared the novel coronavirus (COVID-19) outbreak a global pandemic on March 11, 2020 (3). The COVID-19 pandemic continues to spread rapidly, presenting with a wide range of clinical manifestations from asymptomatic to severe acute respiratory distress syndrome, multiorgan failure and death (4). Initially, the pediatric patients were largely overlooked during the COVID-19 pandemic and were considered a low-risk population, as they accounted for less than 8% of the total cases, and the largest cohort with high morbidity and mortality was reported in the elderly (5–7). In late April 2020, reports from the United Kingdom emerged, describing children who required admission to intensive care units due to an unexplained multisystem inflammatory syndrome with features resembling Kawasaki disease and toxic shock syndrome (8). Subsequently, similarly affected children were reported across Europe and the United States, associated temporally and geographically with COVID-19 outbreaks (4). When an increase in the pediatric COVID-19 cases associated with hyperinflammation was described, the World Health Organization (WHO), the US Centers for Disease Control and Prevention (CDC), and the Royal College of Paediatrics and Child Health (RCPCH) developed overlapping definitions of the syndrome and named it multisystem inflammatory syndrome in children (MIS-C) or paediatric multisystem inflammatory syndrome—temporally associated with SARS-CoV-2 (PIMS-TS or PIMS) (8). These definitions have common elements, such as prolonged fever, multi-organ dysfunction, elevated inflammatory markers, and recent or current SARS-CoV-2 infection or exposure, however, they slightly differ in many other criteria (8–11).

The epidemiology of MIS-C remains unclear, however it appears to be a rare condition with an incidence <1% in children infected with SARS-CoV-2 (12). The mortality and morbidity in those patients differ significantly from the benign course of COVID-19 in children. The majority had previous SARS-CoV-2 infection or known exposure or serologic evidence of SARS-CoV-2, supporting the hypothesis that MIS-C represents an immune-mediated and dysregulated post-infectious inflammatory response possibly triggered by SARS-CoV-2 (13, 14).

Obviously, epidemiological data including incidence of MIS-C and the clinical findings varied significantly across studies, based on the definition used for diagnosis, with broader criteria leading to reports of a higher incidence (8, 15). Despite the risk of overdiagnosis, the true incidence of MIS-C may be significantly higher due to the lack of clinical awareness and specific diagnosis. Furthermore, diagnostic criteria were based on clinical manifestations in children hospitalized with severe disease, which may neglect mild cases (8).

As more cases are described in the literature, the clinical heterogeneity of the disease and its wide spectrum are being further understood. At present, our knowledge of this inflammatory syndrome is incomplete and the available results from published studies provide insufficient insights into the full epidemiological, clinical, immunological, and prognostic spectrum of MIS-C. Therefore, it is pivotal to undergo further studies in order to obtain a better definition of MIS-C, to optimize the characterization and the diagnostic criteria, to assess its true impact and to generate the best clinical and therapeutic approach, in addition to clarifying short term and long-term outcomes.

Following a wave of COVID-19 infections in Lebanon, we encountered several patients suspected to have MIS-C at our tertiary medical center in Lebanon over a 15-month period. Some of these patients presented a diagnostic challenge because they mimicked MIS-C but did not meet the CDC definition (9). We decided to review these cases and describe their features in comparison with cases that met the CDC criteria of MIS-C or those that had an alternative diagnosis.

Materials and methods

Study design

This is a retrospective chart review of patients with suspected or confirmed MIS-C, following current or recent

SARS-CoV-2 infection or exposure to a person with suspected or confirmed COVID-19 infection, who had sufficient or insufficient criteria for diagnosis. The study was conducted at the American University of Beirut Medical Center (AUBMC), located in Beirut, Lebanon, and was approved by the institutional review board (IRB) at AUBMC (IRB ID: BIO-2021- 0090). All patients were identified retrospectively, and their charts were reviewed through medical records by looking at the following ICD-10 codes for admission diagnosis: "Fever of other and unknown origin", "fever" and "multisystem inflammatory disorder". In addition, at least one of the authors on this paper was on the Pediatric Infectious Diseases (PID) service, which is consulted on cases suspected to have MIS-C, for the duration of the study period. Therefore, we used the PID service records to identify any patients that may have been missed by the hospital medical record review.

All children and adolescents <19 years of age, admitted to the hospital between March 1, 2020, to May 31, 2021 were included in the study if they had suspected MIS-C on admission. All included subjects had recent or past infection of SARS-CoV-2 confirmed by real-time reverse-transcriptase -polymerase -chain-reaction (RT-PCR) and/or serology. Notably, COVID-19 vaccines were not available for children and adolescents during the study period.

Subject classification

Included subjects were categorized into the "MIS-C group" when they fulfilled the criteria for MIS-C CDC case definition. Subjects who were thought to have MIS-C upon presentation but did not fulfill the CDC criteria and had no alternative diagnosis, were categorized into the "Near MIS-C" group. The third group "Alternative Diagnosis" included subjects who were initially worked up for MIS-C but ended up with an alternative diagnosis.

In this study, categorization of subjects was based on the CDC case definition for MIS-C throughout the whole analysis, except for the first table of the analysis where subjects were classified according to the three most recognized definitions, the RCPCH, the WHO and the CDC.

Definitions

As the COVID-19 pandemic evolved in different countries, the RCPCH, the WHO and the CDC had developed, based on a limited number of cases, different definitions for the multisystem inflammatory syndrome in children (**Table 1**) (9–11, 16).

Different patterns of clinical presentation and organ system involvement were present in our subjects and ranged from mild to severe forms. Many subjects had mild upper or lower respiratory symptoms such as sore throat, nasal congestion, rhinorrhea or cough, or very mild mucocutaneous symptoms which were not clinically suggestive of significant organ involvement. We described organ system involvement based on the symptoms, clinical findings, and laboratory results (17).

Data collection

The data collection was performed by the study team at the Center of Infectious Diseases Research Center (CIDR). Variables recorded from admission to discharge were extracted from the clinical records assuring anonymity and confidentiality. Variables included demographics, comorbidities, history of COVID-19 infection or exposure to a COVID-19 positive person, presenting signs and symptoms, associated comorbidities, physical examination findings, laboratory tests, imaging reports, viral testing with SARS-CoV-2 RT-PCR and serology tests, clinical course in the hospital, management, and outcomes.

Statistical analysis

Descriptive analyses were performed using numbers and percentages for qualitative variables and means with standard deviation (SD) or median with range for continuous variables. All statistical analyses were performed with the use of the Statistical Package for Social Sciences (SPSS) program, version 25.0 for Windows (Armonk, NY, IBM Corp).

Results

In the 15-month study period, a total number of 29 subjects who presented with a clinical picture suspicious of MIS-C were included in our cohort, with a median age of 5 years.

Based on the RCPCH definition, 21 subjects fit the definition of PIMS (MIS-C), but when the WHO and the CDC case definitions were used, only 15 and 11 subjects, respectively, fit the MIS-C definition (**Table 2**). Most of our subjects were previously healthy; however, 24.1% of them had preexisting medical conditions and comorbidities including hematological malignancies, sickle cell disease, thalassemia, hemophagocytic lymphohistiocytosis (HLH) or obesity. The "Alternative Diagnosis" group included 8 subjects whose workup showed other etiologies including drug-induced pericarditis, salmonella or rotavirus gastroenteritis, adenovirus or rhinovirus/enterovirus infection documented by multiplex PCR respiratory panel, urinary tract infection (UTI), or brucellosis. Overall, there was a male predominance (69%), which was also noted in both MIS-C and "Near MIS-C" groups (81.8% and 80% respectively). Almost 90% of the

TABLE 1 Case definitions for Multisystem inflammatory syndrome in children.

CDC[a]	WHO	RCPCH
An individual aged <21 years presenting with: • Fever >38.0 °C for ≥24 h or report of subjective fever lasting ≥24 h • Laboratory evidence of inflammation, Including, but not limited to, one or more of the following: an elevated CRP, ESR, fibrinogen, procalcitonin, d-dimer, ferritin, LDH, or IL-6, elevated neutrophils, reduced lymphocytes, and low albumin • Evidence of clinically severe illness requiring hospitalization • Multisystem (≥2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic, or neurological) **AND** No alternative plausible diagnoses **AND** Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or exposure to a suspected or confirmed COVID-19 case within the 4 weeks prior to the onset of symptoms	Children and adolescents 0–19 years of age with fever ≥3 days **AND** two of the following: 1. Rash or bilateral non-purulent conjunctivitis or muco-cutaneous inflammation signs (oral, hands or feet). 2. Hypotension or shock. 3. Features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including ECHO findings or elevated Troponin/NT-proBNP), 4. Evidence of coagulopathy (by PT, PTT, elevated d-Dimers). 5. Acute gastrointestinal problems (diarrhoea, vomiting, or abdominal pain). **AND** Elevated markers of inflammation such as ESR, C-reactive protein, or procalcitonin. **AND** No other obvious microbial cause of inflammation, including bacterial sepsis, staphylococcal or streptococcal shock syndromes. **AND** Evidence of COVID-19 (RT-PCR, antigen test or serology positive), or likely contact with patients with COVID-19.	A child presenting with persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia) and evidence of single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) with additional features (see listed in Appendix 1). This may include children fulfilling full or partial criteria for Kawasaki disease[b]. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus (waiting for results of these investigations should not delay seeking expert advice). SARS-CoV-2 PCR testing may be positive or negative

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactic acid dehydrogenase; IL-6, interleukin 6; RT-PCR, reverse transcriptase–polymerase chain reaction; ECHO, echocardiography; NT-proBNP, N-terminal pro–B-type natriuretic peptide; PT, prothrombin time; PTT, activated partial thromboplastin time.
[a]Additional comments: Some individuals may fulfill full or partial criteria for Kawasaki disease[#] but should be reported if they meet the case definition for MIS-C. Consider MIS-C in any pediatric death with evidence of SARS-CoV-2 infection.
[b]Criteria for Kawasaki disease include persistent fever and 4 of 5 principal clinical features: erythema and cracking of lips, strawberry tongue, and/or erythema of oral and pharyngeal mucosa; bilateral bulbar conjunctival injection without exudate; rash (maculopapular, diffuse erythroderma); erythema and edema of the hands and feet and/or periungual desquamation; and cervical lymphadenopathy.

TABLE 2 Cases classification based on different definitions.

	CDC, *n* (%) *N* = 29	WHO, *n* (%) *N* = 29	RCPCH, *n* (%) *N* = 29
MIS-C	11 (37.9)	15 (51.7)	21 (72.4)
Near MIS-C	10 (34.5)	6 (20.7)	0 (0.0)
Alternative diagnoses	8 (27.6)	8 (27.6)	8 (27.6)

subjects had history of recent SARS-CoV-2 infection by RT-PCR or exposure to a suspected or confirmed COVID-19-infected person within the 2 to 8 weeks prior to presentation (Table 3).

Table 4 summarizes the clinical presentation of the subjects. Fever was present in all subjects with a difference in the median duration between the MIS-C group (6 days), the near-MIS-C group (2 days) and the alternative diagnosis group (4 days). Gastrointestinal symptoms were prevalent in the 3 groups. Respiratory symptoms were most encountered in the MIS-C group. When comparing the 3 groups, mucocutaneous involvement was reported in 81.8% of subjects with MIS-C while only 20% and 37.5% of the subjects in the "Near MIS-

TABLE 3 Demographic and clinical characteristics of the patients.

Characteristics	Total, *n* (%) *N* = 29	MISC, *n* (%) *N* = 11	Near-MISC, *n* (%) *N* = 10	Alternative diagnoses, *n* (%) *N* = 8[a]
Median age in years (± SD)	5.0 (1.0–17.0)	8.0 (3.0–17.0)	4.0 (1.0–17.0)	3.0 (1.16–13.0)
Gender				
Male	20 (69.0)	9 (81.8)	8 (80.0)	3 (37.5)
Female	9 (31.0)	2 (18.2)	2 (20.0)	5 (62.5)
Comorbidities[b]	7 (24.1)	4 (36.4)	2 (20.0)	1 (12.5)
History of recent SARS-CoV-2 infection by RT-PCR or exposure to a suspected or confirmed COVID-19 person				
No	3 (10.3)	1 (9.1)	1 (10.0)	1 (12.5)
Yes	26 (89.7)	10 (90.9)	9 (90.0)	7 (87.5)

[a]The alternative diagnoses found in our subjects were pericarditis, salmonella or rotavirus gastroenteritis, adenovirus or rhinovirus/enterovirus infection, urinary tract infection, or brucellosis.
[b]Comorbidities included hematologic malignancies, Sickle cell disease, Thalassemia trait, Hemophagocytic lymphohistiocytosis or obesity.

TABLE 4 Clinical characteristics based on organ system involvement.

Organ system involvement	MISC, *n* (%) *N* = 11	Near-MISC, *n* (%) *N* = 10	Alternative diagnoses, *n* (%) *N* = 8
Respiratory involvement[a]	4 (36.4)	0 (0.0)	2 (25.0)
Gastrointestinal involvement	8 (72.7)	7 (70.0)	6 (75.0)
Mucocutaneous involvement	9 (81.8)	2 (20.0)	3 (37.5)
Cardiovascular involvement[b]	9 (90.0)	0 (0.0)	1 (12.5)
Hematological involvement	7 (63.6)	0 (0.0)	0 (0.0)

[a]6 patients did not have a chest x-Ray or Computed tomography (CT) of the chest performed as they did not have any significant respiratory symptom; one from the MISC group, 2 from the Near-MISC group, and 3 patients from the "Alternative diagnoses" group.
[b]4 patients did not undergo echocardiography, one from the MISC group and 3 from the Near-MISC group.

C" group and "Alternative Diagnosis" group, respectively, had conjunctivitis, cracked lips, strawberry tongue or a rash reported during illness.

Out of 23 subjects who underwent imaging (chest x-ray or chest CT-scan), only 6 subjects had evidence of radiologically confirmed pneumonia, of which 4 subjects belonged to the MIS-C group. Echocardiography was performed in 25 subjects, where 9 out of 10 subjects in the MIS-C group had positive findings including 55.5% with reduced ejection fraction. One of these subjects had severe biventricular dysfunction (ejection fraction <20%) with increased pulmonary artery pressure requiring admission to the pediatric intensive care unit (ICU) and the use of vasopressors. Coronary artery dilation and pericardial effusion were noted, respectively, in one and 4 subjects in the MIS-C group. One subject from the "Alternative Diagnosis" group had moderate pericardial effusion. This subject, known to have hemophagocytic lymphohistiocytosis status four-months post hematopoietic stem cell transplant, showed typical findings of pericarditis for which he received steroids and non-steroidal anti-inflammatory agents. None of the subjects in the "Near MIS-C" group had cardiac involvement. Two subjects with MIS-C developed pleural effusion, whereas none of the subjects with "Near MIS-C" did. None of the subjects had neurological involvement.

Laboratory parameters were reviewed as shown in **Table 5**. Compared with the subjects with "Near MIS-C" and those with alternative diagnoses, subjects with typical MIS-C presentation had higher leukocytosis, platelet counts and acute inflammatory markers, specifically C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin and D-dimer

TABLE 5 Laboratory tests.

Laboratory results	MISC, mean/ (number of tested patients) (±SD)	Near-MISC, mean/ (number of tested patients) (±SD)	Alternative diagnoses, mean/ (number of tested patients) (±SD)
Blood cell counts			
White blood cells (/cu.mm) (*N* = 29)	19,271/11 (±12,058)	9,410/10 (±4,860)	11,975/8 (±4,072)
Platelets (/cu.mm) (*N* = 29)	591,909/11 (±307,002)	291,800/10 (±86,672)	372,437/8 (±235,935)
Inflammatory markers			
CRP (mg/L) (*N* = 29)	176.6/11 (±95.6)	59.4/10 (±45.4)	95.3/8 (±118.2)
ESR (mm/hr) (*N* = 14)	71.4/7 (±34.6)	34.7/3 (±8.3)	35.5/4 (±15.5)
Procalcitonin (ng/ml) (*N* = 8)	6.4/7 (±3.6)	—	0.6/1
Ferritin (ng/ml) (*N* = 28)	909.6/11 (±1,040.8)	557.7/9 (±1,139.1)	137.6/8 (±56.2)
Interleukin-6 (pg/ml) (*N* = 4)	112.7/4 (±153.1)	—	—
Fibrinogen (g/L) (*N* = 25)	4.5/11 (±1.9)	3.6/8 (±0.9)	3.8/6 (±1.5)
D-Dimer (ng/ml) (*N* = 29)	3,038.9/11 (±1,623.3)	661.0/10 (±789.1)	731.7/8 (±287.5)
Cardiac markers			
Pro-BNP (pg/ml) (*N* = 16)	7,507.6/10 (±7,625.0)	268.0/2 (±66.5)	978.0/4 (±1,396.5)
Troponin T (ng/ml) (*N* = 29)	0.055 (±0.069)	0.005 (±0.002)	0.007 (±0.008)
	MISC, n/ number of tested patients (%)	Near-MISC, n/ number of tested patients (%)	Alternative diagnoses, n/ number of tested patients (%)
SARS-CoV2 RT-PCR at presentation (*N* = 29)			
Negative	7/11 (63.6)	9/10 (90.0)	6 (75.0)
Positive	4/11 (36.4)	1/10 (10.0)	2 (25.0)
COVID IgM (*N* = 26)[a]			
Negative	9/10 (90.0)	8/8 (100)	8/8 (100)
Positive	1/10 (10.0)[b]	0/8 (0.0)	0/8 (0.0)
COVID IgG (*N* = 26)[a]			
Negative	1/10 (10.0)[b]	0/8 (0.0)	0/8 (0.0)
Positive	9/10 (90.0)	8/8 (100)	8/8 (100)

[a]COVID antibodies were not tested in 3 patients, one patient who had acute COVID infection from the MISC group and 2 others from the Near-MISC group.
[b]This patient had acute COVID infection, he developed MIS-C almost 11 days after infection.

(Table 5). Elevated troponin and pro-B-type natriuretic peptide (Pro-BNP) levels were mainly detected in the MIS-C group with mean values that were significantly higher than the values reported for the remaining subjects. Among the subjects in the "Near MIS-C" and the alternative diagnoses groups who were serologically tested for SARS-CoV-2, 100% had positive serology test results, mainly IgG, with or without positive RT-PCR test results at one point during their illness whether prior to their presentation or concomitantly. As for the MIS-C group, out of the 11 tested subjects, SARS-CoV-2 IgG was detected in 10 subjects. The remaining subject had positive SARS-CoV-2 IgM and negative IgG as he developed MIS-C around 2 weeks after infection and therefore SARS-CoV-2 IgG did not rise yet.

The outcome and management of patients are shown in Table 6.

None of the 29 subjects required mechanical ventilation or extracorporeal membrane oxygenation. On admission, empiric intravenous broad-spectrum antibiotics were initiated in 91% of the subjects with MIS-C vs. 30% of the subjects with "Near MIS-C". Blood and urine cultures were negative for all subjects. Upon presentation, 3 subjects in the MIS-C group presented with hypotension and/or cardiogenic shock requiring ICU admission and inotropic support. In the MIS-C group, all subjects (100%) were treated with intravenous immunoglobulins (IVIG), 81.8% received methylprednisolone, which was later shifted to oral prednisolone and tapered over 3 to 4 weeks. None of the subjects in the two other groups received IVIG nor vasopressors. Enoxaparin was given to 63.6% of subjects with MIS-C who had severe ventricular dysfunction, dilated coronary arteries, or markedly elevated D-dimer level. The only subject in the "Near MIS-C" group who received enoxaparin had a hematological malignancy and markedly elevated D-dimer level. No mortality was documented, all subjects recovered.

TABLE 6 Complications, outcome and management.

Complications, Outcome and Management	MISC, *n* (%) *N* = 11	Near-MISC, *n* (%) *N* = 10	Alternative diagnoses, *n* (%) *N* = 8
ICU admission	3 (27.3)	0 (0.0)	0 (0.0)
Length of stay (in days), median (range)	8.0 (1.0–22.0)	3.0 (1.0–5.0)	5.0 (3.0–19.0)
Treatment (*N* = 29)			
Antibiotic therapy	10 (90.9)	3 (30.0)	4 (50.0)
IVIG[a]	11 (100)	0 (0.0)	1 (12.5)
Steroids	9 (81.8)	1 (10.0)	1 (12.5)
Aspirin	10 (90.9)	2 (20.0)	1 (12.5)
Enoxaparin	7 (63.6)	1 (10.0)	0 (0.0)
Vasopressors	3 (27.3)	0 (0.0)	0 (0.0)

[a]IVIG, intravenous immunoglobulins.

Illustrative cases

The clinical course of two representative subjects is shown in Figures 1, 2, respectively. The first subject is a 4-year-old patient with thalassemia trait who returned from a trip to Turkey 3 weeks prior to presentation. He presented to our center with high-grade fever of 6 days duration, nasal congestion, and rash. Two nasopharyngeal RT-PCR tests for SARS-CoV-2 in the preceding few days were negative. On physical examination, he had bilateral nonexudative conjunctivitis with limbic sparing and progressive erythematous maculopapular blanching rash over his upper and lower extremities. Laboratory work up was significant for leukocytosis (26,000/mm^3) with neutrophil predominance, microcytic anemia, low albumin, prolonged prothrombin time and International Normalized Ratio (INR), and elevated inflammatory markers including CRP, procalcitonin, ferritin and D-dimer. His SARS-CoV-2 serology yielded a positive IgG indicating a previous infection. Echocardiography showed mildly reduced ejection fraction, prominent coronary arteries, as well as mild tricuspid and mitral regurgitation. He was initially hemodynamically unstable requiring admission to the ICU and inotropic support. He was started empirically on broad spectrum antibiotics (vancomycin and ceftriaxone). The patient was diagnosed with MIS-C based on the CDC case definition and received IVIG, steroids and low-dose aspirin.

The second subject is a 12-month-old boy who presented to the emergency department with a 2-day history of high-grade fever, associated with vomiting, decreased oral intake and urine output. His physical examination was remarkable for pharyngeal exudates, but no cervical adenopathy. The patient's parents had COVID-19 infection around 3 weeks prior to the onset of his symptoms, during which the patient had one episode of high-grade fever and diarrhea but was not tested. COVID-19 RT-PCR done on admission was negative while SARS-CoV-2 serology yielded a positive IgG and negative IgM. He had a WBC count of 13,900 /mm^3 and CRP level of 112.7 mg/L. He was admitted to the pediatric unit for intravenous hydration and was started on aspirin as his echocardiography revealed prominent distal left anterior descending (LAD) coronary artery, with good biventricular function, and no pericardial effusion. This patient did not meet the full criteria for MIS-C and therefore was considered as a case of "Near MIS-C". It is remarkable to mention that white count, platelet count, and inflammatory markers improved significantly at two, five, and twelve days later. In addition, the prominent distal LAD improved gradually on follow up echocardiography evaluations performed at one, three, and five months. However, it is worth mentioning that

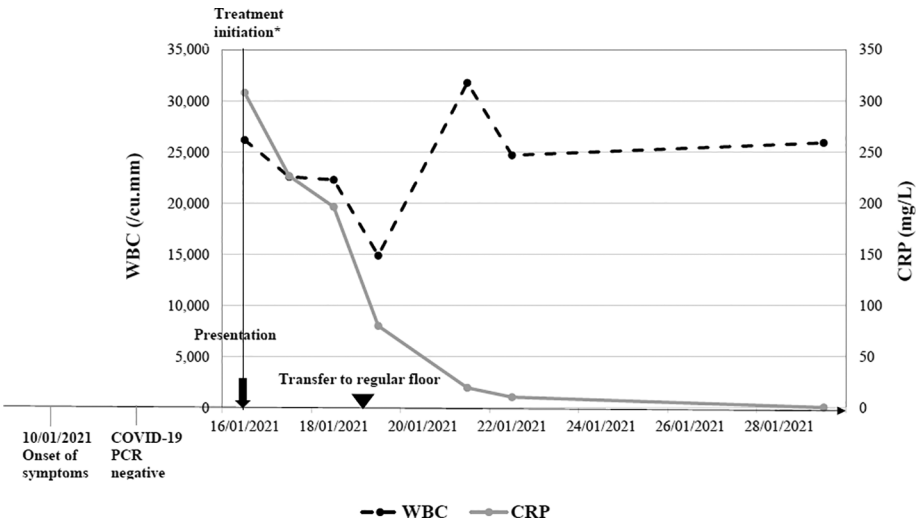

FIGURE 1
Clinical course of a 4-year-old with confirmed MIS-C. *Admission to Pediatric Intensive care unit and treatment administration including IVIG, steroids, aspirin and antibiotics.

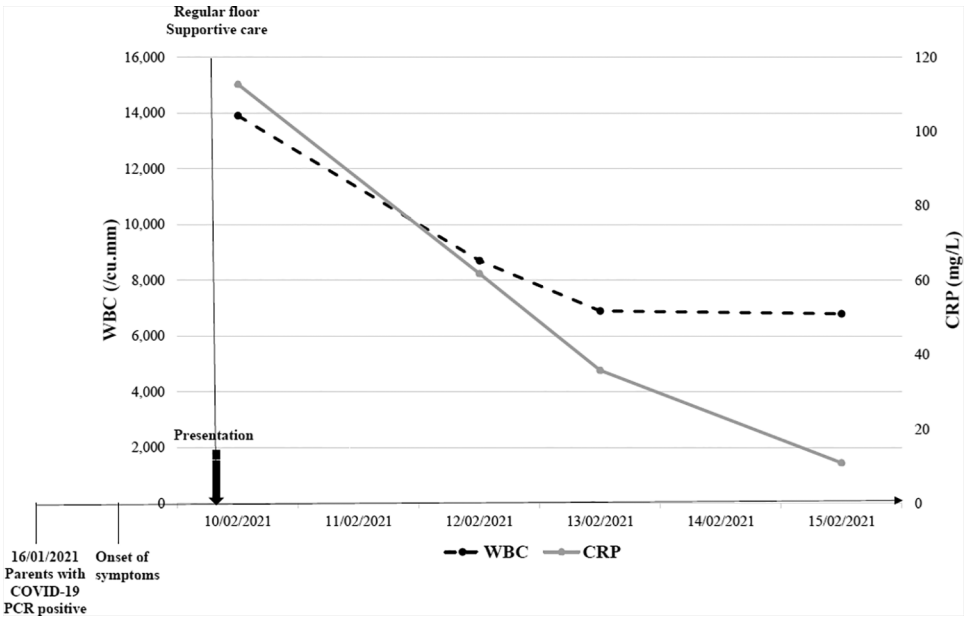

FIGURE 2
Clinical course of a patient with near MIS-C.

no further investigations to rule out a possible viral etiology were done since the patient clinically improved within two days without substantial intervention and that the initial presentation did not qualify for the diagnosis of Kawasaki disease (only 2 days of fever, no lymphadenopathy, mucositis, rash and never developed desquamation, or laboratory findings of thrombocytosis or hypoalbuminemia).

Discussion

In the current series of patients with, or suspected to have, MIS-C, fever was present in all subjects including those from the "Near MIS-C" and "Alternative Diagnosis" groups. Cardiovascular system involvement was the most reported in our MIS-C group followed by mucocutaneous and

gastrointestinal systems involvement. A recent systematic review also showed that cardiovascular and gastrointestinal symptoms were most commonly seen in MIS-C patients (15), in addition an Italian single-center study reported that 81% of their MIS-C patients had cardiac involvement, confirming that the heart along with the gastrointestinal system are most involved in MIS-C patients (18). Gastrointestinal symptoms were similarly encountered in the "Near MIS-C" and "Alternative Diagnosis" group but none had clinical evidence of coagulopathy or cardiac abnormalities except for one patient who was suspected to have cyclosporin-induced pericarditis. Hence, subjects with "Near MIS-C" did not meet the CDC case definition criteria but were hospitalized for close monitoring lest they develop additional organ involvement after their initial presentation.

MIS-C presents a diagnostic challenge for the physician due to the nonspecific early symptoms, lack of pathognomonic findings, and potentially fatal complications. Our understanding of the pathogenesis of this syndrome is evolving but remains limited. Whereas genetic predisposition to developing severe MIS-C has been demonstrated (19), the true triggers for the severe inflammatory response seen in otherwise healthy individuals with MIS-C and characterized by increased plasma cytokine levels including IL-6, IL-8, IFNγ, IL-17, TNFα, and IL-10 and the multilineage immune cell activation remain unknown (20–24). A specific immunopathologic signature and association with HLA alleles were identified recently that may pave the way for the development of more specific biomarkers (25). Importantly, the magnitude of the inflammatory response in MIS-C correlates with disease severity (23) and this raises the possibility that this syndrome is actually a continuum of clinical severity rather than an all-or-none response as the current definitions seem to imply.

In the present study, we compared the RCPCH, the WHO and the CDC case definitions (9–11). We found that the RCPCH definition was more inclusive and identified all subjects with MIS-C and "Near MIS-C" and excluded those who had features of MIS-C but were found to have an alternative diagnosis. The WHO and the CDC definitions are more stringent concerning clinical manifestations and the relationship with SARS-CoV-2 infection. In our case series, the WHO definition identified more subjects in the MIS-C group compared to the CDC definition, with few subjects whom we classified as "Near MIS-C" as they did not present multisystem dysfunction or did not have persistent fever for ≥3 days as per the WHO definition. In contrast to that of the WHO, the CDC definition excluded few subjects of those included in the MIS-C group based on the WHO definition, all failing to achieve the CDC criterion concerning the multi-organ (≥2 organ systems) dysfunction. Similarly, in a systematic review, Hoste et al. observed that although the RCPCH definition included all cases, nevertheless both the CDC and WHO case definitions were more selective, requiring multisystem involvement and a proven association with SARS-CoV-2, with the WHO definition including 97% of cases and the CDC definition 62% (15). Clearly a more precise definition for MIS-C based on a better understanding of the pathogenesis and the identification of more specific clinical and laboratory markers is needed.

The dynamic nature of this syndrome presents a further challenge to the physician. In the literature, several cases were reported to present with incomplete criteria initially but to later develop typical findings including cardiac involvement (16, 19). This has led us to hospitalize patients when the diagnosis of MIS-C was highly suspected, to provide close monitoring and rapid intervention when needed. This is further confounded by the frequent presence of another infection at the time of presentation. All the current definitions include the lack of "alternative plausible diagnoses (CDC)" (9), "other obvious microbial cause of inflammation (WHO)" (11), or "any other microbial cause (RCPCH)" (10). In our series, eight subjects ended up with an alternative diagnosis: drug-induced pericarditis, UTI, respiratory tract infections caused by adenovirus or rhinovirus/enterovirus, or infection with brucella, salmonella, or rotavirus. In these cases, the suspicion of MIS-C arose due to the temporal association with COVID-19 infection or exposure and the presence of commensurate clinical and laboratory findings including elevated inflammatory markers and D-dimers. In this regard, several questions present themselves for treating physicians: (1) how extensively should they work up the patient looking for an alternative diagnosis? (2) once identified, is the alternative diagnosis sufficient to explain the clinical presentation? (3) are the laboratory findings in keeping with the alternative diagnosis, e.g., does a rhinoviral infection explain the significantly elevated D-dimer level? Finally, (4) does the identification of an alternative diagnosis rule out the possibility of concomitant MIS-C? These questions present dilemmas for the treating physician who must decide on further treatment but has no discernible answers. An extensive work up to exclude a microbial infection may be necessary to rule out the majority of the alternative diagnoses, e.g., multiplex PCR respiratory and gastrointestinal panels that may not be readily available at most hospitals especially in underprivileged locations. Moreover, these additional tests present a significant economic burden on the healthcare system. On the other hand, not performing the extensive work up may lead to overdiagnosis of MIS-C as patients may satisfy all the criteria in the absence of an alternative diagnosis as reported previously (26).

Whether the identification of an alternative diagnosis is sufficient to explain the clinical and laboratory findings is not always straightforward. For example, all our subjects

demonstrated elevation in inflammatory markers specifically CRP, ESR and D-dimer, which are nonspecific and overlap with many other infectious and inflammatory diseases (27). In the pre-COVID-19 era, and before the emergence of MIS-C, obtaining laboratory values such as ferritin and D-dimer for evaluation of febrile children was uncommon. In pediatric patients, D-dimer is requested for a select group of diagnoses including pulmonary embolism, disseminated intravascular coagulation, or hyperthrombotic states (28), however this is not the practice for other causes such as rhinovirus, rotavirus, or salmonella infections. For that reason, expected values for such tests in children with common febrile illnesses remain unknown (27). This is illustrated in the current series where we could not be certain whether the alternative diagnoses found in some of our subjects were associated intrinsically with elevated D-dimer or this finding was due to concomitant MIS-C or a recent COVID-19 infection. Not performing the D-dimer level doesn't seem to be a viable option as an increased level is sometimes the only clue for MIS-C and coronary artery dilatation (29). Thus, trending these inflammatory markers in subjects with suspected MIS-C, even in the presence of an alternative diagnosis, may help identify concomitant diagnoses of MIS-C, prevent disease progression, and multi-organ involvement (30).

Clearly, more research is needed to fully understand the pathogenesis of MIS-C and to fully appreciate its clinical presentation spectrum. Based on our experience reported in the current series, we favor the hypothesis that MIS-C has a continuum of severity where the most severe cases present as Kawasaki disease-like picture or shock/myocarditis and the less severe cases present with various degrees of fever and hyperinflammation. The current study is limited by its retrospective nature from a single center. As a result, not all cases suspected to have MIS-C were worked up to the same extent. Thus, larger prospective studies are required to verify this hypothesis to provide unambiguous answers. For example, current databases recruiting MIS-C patients (31–33) should expand to include subjects with "Near MIS-C" and even those with an alternative diagnosis that may not fully explain all the clinical and laboratory findings. This is going to be more important as the pandemic wanes and the disease becomes more sporadic presenting an even more difficult diagnostic challenge for treating physicians.

Data availability statement

The datasets presented in this article are not readily available because of privacy and ethical restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

Ethical clearance was obtained from the Institutional Review Board (IRB) at the American University of Beirut Medical Center (BIO-2021-0090) in line with the World Medical Association, Declaration of Helsinki in 2013. A waiver from obtaining consent from subjects was granted by IRB for this study since we were reviewing medical records retrospectively, all data collected were stripped of patient identifiers, and there is no risk to subjects.

Author contributions

SK, NY, RHW and GD contributed to conception and design of the study. SK, NY, SBK, NAH, RS, DH, NEM, ON, AB and GBS collected data. SK and CB performed the statistical analysis and participated in the results writing. SK, NY wrote the initial draft of manuscript. ZEZ wrote sections of the manuscript. RHW and GD critically revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Feasibility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen self-testing in school and summer camp attendees

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Background: SARS-CoV-2 screening is one of the pillars of non-pharmaceutical preventive strategies to early identify and isolate infected individuals and therefore decrease community incidence.
Methods: We assessed the feasibility of severe acute respiratory syndrome coronavirus 2 self-testing with antigen-detecting rapid diagnostic tests in attendees of educational settings.
Results: A total of 305 students (88.15%) and 41 staff (11.85%) from 9 to 56 years old participated in the self-testing procedure and answered the survey at the end of the study. 91.3% ($n = 313$) did not need help, 96.1% of participants reported the same outcome as the healthcare workers. 94.5% strongly or slightly agree with the statement "I would repeat the experience".
Conclusion: The study demonstrates that self-testing is acceptable and usable in children, adolescents and adults when the epidemiological situation may require a systematic screening of these populations, although supervision by health care or previously trained personnel is recommended for younger age groups.

KEYWORDS

acceptability and usability, SARS-CoV-2 antigen testing, SARS-CoV-2, school public health, self-testing

Introduction

Since the emergence of SARS-CoV-2 in December 2019 governments have implemented various measures to control the spread of the virus. Schools were severely affected and initially closed in many countries, including Spain, despite uncertainty if school closures were an effective containment measure, with a negative impact on the education of children and adolescents (1).

However, data from different countries showed that reopening or never closing schools was not necessarily associated with a significant increase in child-to-child or community transmission in children under 14 years of age (2).

Measures must be adapted to each setting to prevent transmission of the virus (3). An important strategy to minimize SARS-CoV-2 transmission is the rapid identification of infected people, symptomatic or not. Non-pharmaceutical preventive interventions such as screening asymptomatic people for SARS-CoV-2, have been shown to decrease incidence at the community level (3). Antigen detection rapid diagnostic tests (Ag-RDTs) have been proposed as suitable for point-of-care screening of potentially exposed people. Advantages of Ag-RDTs include: low price, absence of referral to a high-tech laboratory, short turnaround time for results and identification of people with potential to transmit their SARS-CoV-2 infection (3) However, taking into account that rapid tests are often developed and marketed when outbreaks are already advanced, should be considered as a medium to long term measure. Making Ag-RDTs available in educational centers could reduce care school closures, costs, response time and eventually SARS-CoV-2 transmission.

As nasal Ag-RDT self-testing is considered to be reliable and feasible in adults (4, 5), the main objective of this study was to assess whether it is acceptable and usable in younger ages, by focusing on students and staff in schools and summer camps during the fifth wave caused by the Delta B.1.617.2 variant of SARS-CoV-2 in Catalonia (Spain).

Materials and methods

Study population and period

The research took place between April and August 2021 in 2 schools [one from COVID-19 Sentinel Schools Network of Catalonia (6) and other school from ESCORAT project] and 4 summer camps in Catalonia, Spain. Thus, here students over 9 years old were invited to participate and staff were included only in summer camps. In one school (School A) a prospective cohort was established (testing weekly for 8 weeks) while a cross-sectional study was done in School B and summer camps. The field team consisted of between 2 and 4 healthcare workers (nurses and nursing assistants).

Material delivery and data collection

After signing the informed consent, participants received a sampling kit including: paper-based schematic and illustrated instructions for the self-testing procedure, a printed feasibility survey, ID labels and the SARS-CoV-2 Ag-RDT Kit [test cassette, nasal swab, empty tube and plug, Pasteur pipette and buffer solution; Panbio™ COVID-19 Ag Rapid Test Device (Abbot Laboratories, Chicago, US)]. Additionally, the self-test procedure was recorded on video and distributed to participating schools.

Tests were performed in accordance with biosecurity measures (well-ventilated area, separation between participants of >1.5 m, table disinfection with alcohol before and after the procedures). Subsequently, acceptability surveys were entered into the EUSurvey platform by the research team.

All test results were validated. In School B and summer camps, participants read the test result themselves, and the healthcare team validated the reliability, while in School A previously trained older pupils (15–16 years old) supervised the sampling procedure and read the results.

Ag-RDT Positive cases were referred to the health center for a Reverse Transcription-PCR (RT-PCR) with nasal swab to confirm the results.

Feasibility evaluation

We assessed the acceptability and usability of the intervention among participants based on a conceptual validated framework adapted from previous studies (7). Here, acceptance and use of SARS-CoV-2 Ag-RDT self-testing was adapted and divided into the following subdomains: *Learnability, Willingness, Suitability, Satisfaction and Efficacy* (see **Table 1**).

A semi-structured interview was conducted with trained pupils who read the test results (School A) to assess the acceptability and usability of the experience, this was recorded and later transcribed for analysis.

Data analysis

A descriptive analysis of the sample was carried out and the percentages of the categorical variables were calculated for each category, stratifying by age group in accordance with the educational stages of Catalonia based on Law 12/2009 of 10 July 2009 on education. The *p*-value was obtained by means

TABLE 1 Acceptability and usability subdomains results by age groups. *N* = 346, April-August 2021, Catalonia (Spain).

				Participants by age range				
			Total *N* (%)	9–11 years old *N* (%)	12–15 years old *N* (%)	16–18 years old *N* (%)	19–56 years old *N* (%)	*p*-value[a]
ACCEPTABILITY & USABILITY[c]	**Learnability**	Has anyone helped you to take the self-test?						<0.001
		Yes	30 (8.7%)	12 (27.9%)	18 (13.1%)	0 (0.00%)	0 (0.00%)	
		No[b]	313 (91.3%)	31 (72.1%)	119 (86.9%)	124 (100%)	39 (100%)	
		Were you able to complete the self-test successfully?						1.000
		Yes	340 (99.7%)	43 (100%)	137 (99.3%)	121 (100%)	39 (100%)	
		No[b]	1 (0.3%)	0 (0.00%)	1 (0.72%)	0 (0.00%)	0 (0.00%)	
		How easy or difficult did you find it to take the COVID-19 rapid antigen self-test?						0.075
		Very easy + Slightly easy	301 (87.8%)	35 (83.3%)	118 (84.3%)	109 (89.3%)	39 (100%)	
		Neither easy nor difficult	38 (11.1%)	7 (16.7%)	19 (13.6%)	12 (9.84%)	0 (0.00%)	
		Slightly difficult + Very difficult	4 (1.1%)	0 (0.00%)	3 (2.14%)	1 (0.82%)	0 (0.00%)	
		Reading success						0.215
		Same outcome for participants vs. *health workers*	270 (96.1%)	32 (94.1%)	85 (93.4%)	114 (97.4%)	39 (100%)	
		Different outcome for participants vs. *health workers*	11 (3.9%)	2 (5.88%)	6 (6.59%)	3 (2.56%)	0 (0.00%)	
	Willingness	How much do you agree or disagree with the following statement: "I would repeat the COVID-19 antigen rapid self-test in the future"?						0.136
		Strongly agree + Slightly agree	310 (94.2%)	34 (89.5%)	119 (91.5%)	120 (98.4%)	37 (94.9%)	
		Neither agree nor disagree	16 (4.86%)	3 (7.89%)	9 (6.92%)	2 (1.64%)	2 (5.13%)	
		Slightly disagree + Strongly disagree	2 (0.61%)	1 (2.63%)	1 (0.77%)	0 (0.00%)	0 (0.00%)	
		Other	1 (0.3%)	0 (0.00%)	1 (0.77%)	0 (0.00%)	0 (0.00%)	
		Would you be willing to repeat this self-antigen test twice a week if the epidemiological situation requires it?						0.008
		Yes	292 (94.5%)	29 (87.9%)	113 (91.1%)	113 (98.3%)	37 (100%)	
		No	17 (5.5%)	4 (12.1%)	11 (8.87%)	2 (1.74%)	0 (0.00%)	
		If you had to repeat the COVID-19 antigen test, you would prefer to do it in:						<0.001

(*continued*)

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TABLE 1 Continued

				Participants by age range				
			Total *N* (%)	9–11 years old *N* (%)	12–15 years old *N* (%)	16–18 years old *N* (%)	19–56 years old *N* (%)	*p*-value[a]
		Health care centre	55 (17.7%)	14 (35.0%)	24 (18.6%)	13 (12.0%)	4 (11.8%)	
		At home	224 (72.0%)	22 (55.0%)	84 (65.1%)	88 (81.5%)	30 (88.2%)	
		Others	32 (10.3%)	4 (10.0%)	21 (16.3%)	7 (6.48%)	0 (0.00%)	
	Suitability	How much do you agree or disagree with the following statement: "I am confident that the reading of the result I have made is correct"?						0.030
		Strongly agree + Slightly agree	254 (92.4%)	29 (85.3%)	76 (89.4%)	114 (97.4%)	35 (89.7%)	
		Neither agree nor disagree	20 (7.3%)	5 (14.7%)	8 (9.41%)	3 (2.56%)	4 (10.3%)	
		Slightly disagree + Strongly disagree	1 (0.3%)	0 (0.00%)	1 (1.18%)	0 (0.00%)	0 (0.00%)	
		How much do you agree or disagree with the following statement: "I trust this type of self-test"?						0.465
		Strongly agree + Slightly agree	241 (86.7%)	31 (93.9%)	76 (84.4%)	102 (87.9%)	32 (82.1%)	
		Neither agree nor disagree	30 (10.8%)	2 (6.06%)	12 (13.3%)	12 (10.3%)	4 (10.3%)	
		Slightly disagree + Strongly disagree	7 (2.50%)	0 (0.00%)	2 (2.22%)	2 (1.72%)	3 (7.69%)	
	Satisfaction	What is your assessment of the experience of taking the COVID-19 antigen rapid self-test?						0.099
		Very satisfied + Slightly satisfied	307 (91.1%)	36 (90.0%)	119 (86.9%)	113 (93.4%)	39 (100%)	
		Neither satisfied nor dissatisfied	28 (8.31%)	4 (10.0%)	17 (12.4%)	7 (5.79%)	0 (0.00%)	
		Slightly dissatisfied + Very dissatisfied	2 (0.59%)	0 (0.00%)	1 (0.73%)	1 (0.83%)	0 (0.00%)	
		How much do you agree or disagree with the following statement: "I would recommend the COVID-19 rapid antigen self-test to a friend"?						0.774
		Strongly agree + Slightly agree	266 (94.0%)	33 (94.3%)	85 (93.4%)	112 (94.9%)	36 (92.3%)	
		Neither agree nor disagree	15 (5.3%)	2 (5.71%)	5 (5.49%)	6 (5.08%)	2 (5.13%)	
		Slightly disagree + Strongly disagree	2 (0.7%)	0 (0.00%)	1 (1.10%)	0 (0.00%)	1 (2.56%)	

[a]*p*-value calculated using chi-square test.

[b]Reasons for those who need help or did not succeed on sampling are included as a text in the results section of the manuscript.

[c]The *Efficacy* subdomain is not included in the table because is defined as the ability to create a network with the main actors of the local health and education system -primary care, epidemiological surveillance service, educational community, local political agents-, correct communication of results, management of positive cases according to governmental protocols, and appropriate waste management, and results are presented in the aforementioned section.

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of a Chi-square or Fisher test when the frequency was less than 5 using R software (version R-4.0.5).

Ethics

Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol) (code 20/192-PCV) and the Ethics and Clinical Research Committee of the Hospital Universitari Germans Trias (code PI-21-057), approved the study. Informed consent was obtained from all participants.

Results

A total of 305 students (88.15%) and 41 staff (11.85%) from 9 to 56 years old participated in the self-testing procedure and answered the survey at the end of the study. 207 were women (60%), 132 were men (38,3%), 4 non-binary people (1,16%), 2 transgender women (0,58%) and one participant with no information. Data was grouped in four age groups: 9–11 ($n = 43$; 12,4%), 12–15 ($n = 140$; 40,5%), 16–18 ($n = 124$; 35,8%), 19–56 ($n = 39$; 11,3%) (Figure 1).

Acceptability and usability subdomain results of the survey are presented in Table 1. Regarding the *Learnability* subdomain, 91.3% ($n = 313$) did not need help (Table 1), and those needing help mainly chose: *I was too nervous* ($n = 14$; 48,3%), *I needed help adding the buffer solution* ($n = 2$; 6,9%), *I needed help reading the result* ($n = 2$; 6,9%) or *Other reasons* ($n = 9$; 31%) (data not shown).

Regarding advantages, most participants identified: *Results within minutes* ($n = 245$; 86.0%), *Testing at school instead of at a health center* ($n = 244$; 85.6%) and *Tests improve safety and protection against Covid* ($n = 224$; 78.6%); followed by others such as *I can take control of my health with respect to Covid* ($n = 191$; 67.0%), *It gives me a more relaxed feeling when meeting friends* ($n = 181$; 63.5%), *Contributes to the normalization of Covid tests* ($n = 164$; 57.5%) and *The test is free of charge* ($n = 160$; 56.1%). Regarding disadvantages, the majority of participants identified that the test is *Less reliable than RT-PCR* ($n = 213$; 76.9%), while a few considered *You have to interpret the result yourself* ($n = 48$; 17.3%), or *Not having the emotional and/or logistical support to read the result* ($n = 17$; 6.14%) as disadvantages.

Regarding test reading, 96.1% of participants ($n = 270$) reported the same outcome as the healthcare workers (School B and summer camps). 11 participants (3.91%) scored a

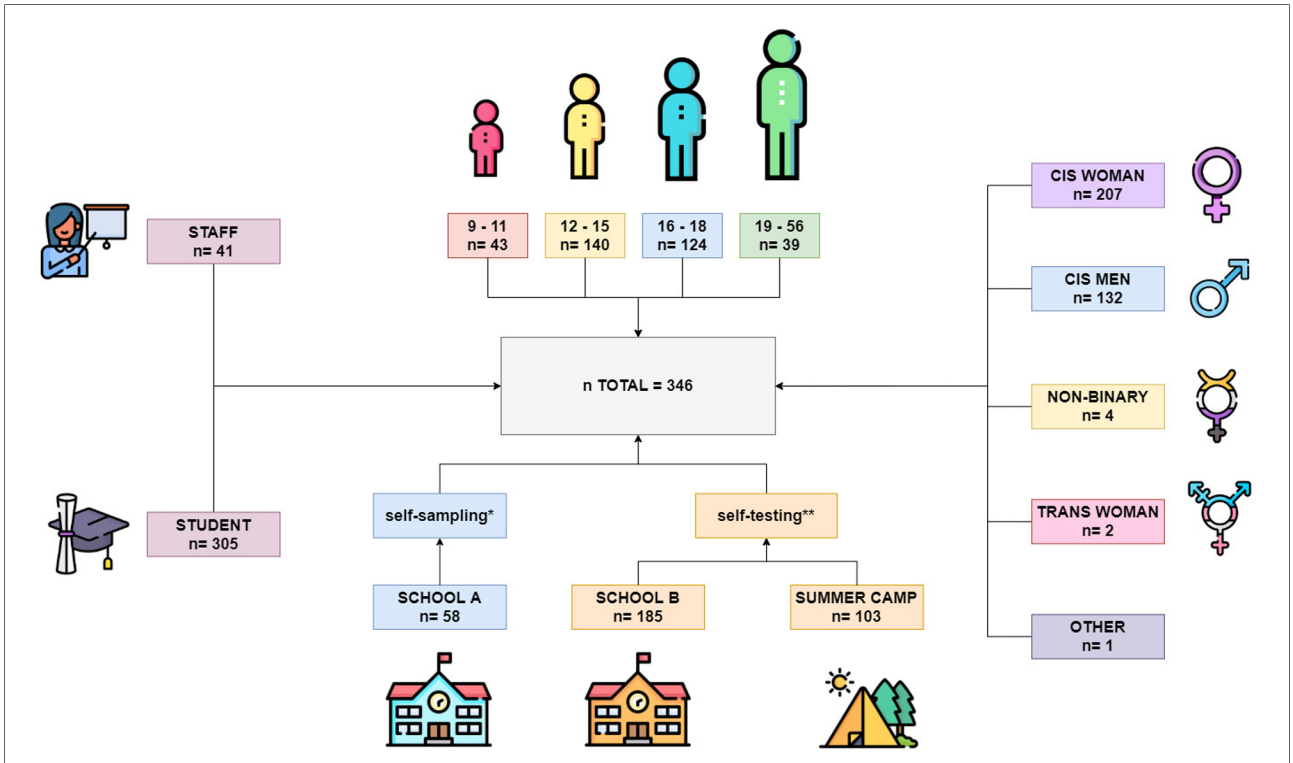

FIGURE 1
Summary of the total number of participants in the pilot study, Spain, N: 346. *Self-sampling: In school A, older pupils (15–16 years old) were trained beforehand to read the results of the other participants. ** Self-testing: In School B and summer camps, participants read the test result themselves, and the healthcare team validated the reliability.

different outcome to the healthcare workers. Of these, 9 students (81,1%) indicated a negative result when the healthcare worker said positive, and 2 students (18,2%) answered that *they don't know the result,* when healthcare worker recorded a negative result.

In terms of *Efficacy*, there was a rapid response and proper management of positive cases, both at the educational and public health levels.

Through the semi-structured interview, the students of School A reported a *positive feeling about the research project*; that *the study helps to have a safer school* and *that a weekly test is acceptable*. However, trained students highlighted the heavy workload, with loss of class hours, and suggested involvement of more students in the future.

Discussion

Overall, the study demonstrates high acceptability and usability of nasal swab Ag-RDT self-tests in students and staff in our settings. To our knowledge no previous results on feasibility in-depth account of acceptability and usability in younger groups in educational settings exist, although studies on specificity and sensitivity of this method do (8). Our results for adults (students and staff) agree with previous studies with rapid antigen and antibody tests (5, 9).

The results suggest that self-testing should be done under health workers or trained individuals' supervision in participants under 15 years of age; they needed more help and were less willing to repeat the test. Younger participants were also less confident about reading the result. In contrast to other studies, no statistical differences were found between age groups when reading the Ag-RDT result (10).

In addition, there was a high (more than 90%) agreement between self-reported and health care worker validated test readings.

The results also show that participants of all ages perceive the turnaround time and not having to travel to a health center as positive elements of the experience. It has been suggested that Ag-RDT self-testing can be a good tool for monitoring outbreaks, avoiding health care bottlenecks and improving access to diagnosis in places with less access to PCR laboratory tests. It also puts the patient at the center of the management of an infectious disease (3, 11). However, risk must be considered in the actions taken by each individual in the event of a positive case in relation to existing public health measures. Also, in an epidemic context, rapid tests are often developed and made commercially available at late stages, and therefore have to be considered as a medium to long term tool for outbreak management.

The study has some limitations to be noted. The number of participants was small -especially with regard to the representation of certain age groups- it is based on an opportunistic sample, and it would be interesting to include students of younger ages.

Due to the current massive use of Ag-RDT in different countries at the domestic level, more studies on feasibility of home-based testing in children, both self-testing and family testing are needed.

Conclusions

The study demonstrates that SARS-CoV-2 antigen self-testing with nasal swabs is acceptable and usable for implementation in schools and summer camps with students and staff, when the epidemiological situation may require a systematic screening of these populations as demonstrated during the 5th Covid wave in Spain. These data have public health implications and remain of interest in case of emergence of new SARS-CoV-2 variants or other potential infectious agents. The strategy would relieve health centers work load, reducing the time it takes travel to health care centers for the educational community. However, in younger age groups supervision by healthcare or other previously trained in-school personnel is recommended.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The studies involving human participants were reviewed and approved by Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol) (code 20/192-PCV) and the Ethics and Clinical Research Committee of the Hospital Universitari Germans Trias (code PI-21-057), approved the study. the participants older than 16 years old or the participants' legal guardian of participants under 16 provided their written informed consent to participate in this study. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CA, JC and BR: contributed to the conceptualisation of the study and funding acquisition. ACC, HMR, AB, LAG, MMF, PRdG, JVA, ESS, JML, BR, JC and CA: contributed to the investigation. LAG and MMF: were responsible for data

analysis. ACC and HMR: were responsible for writing the original draft. All authors contributed to the article and approved the submitted version..

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics of pediatric COVID-19 infections and the impact of influenza and COVID-19 vaccinations during the first two years of the pandemic

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The data regarding the demographics of SARS-CoV-2 in the pediatric population has been published based on several single-center experiences or on metanalyses over short time frames. This article reports data on the demographics of pediatric patients with COVID-19 on a global scale using the TriNetX COVID-19 Research Network. In addition, we examined the risk of COVID-19 infection in relation to the body mass index (BMI) category and the protective value of influenza and COVID-19 immunization against COVID-19 infection. The incidence of COVID-19 infection was higher in the younger age group (≤6 years old), but no gender differences. The incidence of COVID-19 infection was higher among African Americans/Black race (28.57%) White race (27.10%), and obese patients; across all age groups, all genders, all races, and ethnicities ($p < 0.0001$). The incidence of MIS-C was also higher in patients with obesity (OR 1.71, CI 1.36–2.14). We found that the patients who were neither vaccinated for COVID-19 nor influenza within one year before their COVID-19 diagnoses compared to those who received influenza vaccine only, had significantly higher odds for hospitalization (OR 1.19, CI 1.18–1.21), development of MIS-C (OR 1.52, CI 1.32–1.74), and more importantly mortality (OR 1.47, CI 1.26–1.71). In addition, those patients who were neither vaccinated for COVID-19 nor influenza within one year before their COVID-19 diagnoses, compared to those who received at least one dose of COVID-19 vaccine, had significantly higher odds for hospitalization (OR 1.11, CI 1.04–1.19). However, those patients who did not receive the influenza vaccine within one year before their COVID-19 diagnoses nor received the COVID-19 vaccine had much higher odds for hospitalization (OR 1.46, CI 1.41–1.51), MIS-C (OR 3.72, CI 2.11–6.56), and mortality compared to those who received both vaccinations (OR 13.55, CI 1.91–9.62). Using the multiplicative interaction scale, we found a positive interaction between the COVID-19 vaccine and the influenza vaccine; they both combined have a larger effect than each separately. Our study is the largest of its kind (to date) examining the global demographic of the pandemic and the first of a kind to find a link between influenza vaccine and COVID-19-related hospitalization, MIS-C, and mortality in the pediatric population.

KEYWORDS

coronavirus, COVID-19, multisystem inflammatory syndrome in children (MIS-C), body mass index (BMI), obesity, influenza, vaccine

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spanned the earth in less than four months from its inauguration in Wuhan, China, in December 2019, resulting in the World Health Organization (WHO) announcing it as a pandemic on March 11, 2020 (1). To date, approximately 565 million individuals (resulting in more than six million deaths, in 215 countries, areas, or territories have been affected (2). As of July 22, 2022, more than eighty-nine million cases of COVID-19 have been confirmed in the USA (3). A recent report from the American Academy of Pediatrics and the Children's Hospital Association estimated nearly 13.5 million COVID-19 cases among children, with 0.1%–1.5% of these children being hospitalized, and a very low mortality rate (<0.02%) (3). Previous studies have shown that age, gender, and race/ethnicity are independent risk factors for poor outcomes in COVID-19 (4–7). Obesity was reported as an independent risk factor for critical illness in pediatric patients with COVID-19, and this association was modified by age (8). Also, obese communities were found among the best predictors of COVID-19-related deaths (9). The goal of our work is to describe—at a significantly larger scale—the distribution of COVID-19 among different pediatric age groups, races, ethnicities, BMI categories, and the relation between multisystem inflammatory syndrome in children (MIS-C) incidence (being one of the severe COVID-19 complications) and BMI, as well as the relationship between prior influenza and/or COVID-19 vaccination, and hospitalization, MIS-C, and mortality. Understanding how these factors play a role in infection with SARS-CoV-2 is crucial for patient risk stratification and care, and will potentially enlighten researchers and clinicians about which children are at higher risk for developing COVID-19 infection and its secondary complications.

Methods

In this retrospective cohort analysis, data analysis was performed using the TriNetX COVID-19 Research Network. TriNetX is a real-world platform of aggregated electronic health records (EHRs) from seventy-five health care organizations (HCOs) globally, which has a total of 17,158,646 pediatric patients ages 18 years or less. Two-thirds of the TriNetX patients are from the US and one-third from outside the US including (~10% Latin America, ~10% Europe, and ~10% Asia). We created queries using the TriNetX's analytics function to analyze the outcomes of interest among children up through 18 years old, between December 2019 till July 22, 2022. The outcomes of interest included (1) COVID-19 infection among different age groups, genders, races, ethnicities, and BMI categories, (2) incidence of MIS-C among different age groups and different BMI categories, and (3) odds of hospitalization, MIS-C, and mortality among COVID-19 patients according to their COVID-19/influenza vaccination status. To examine the association between exposure and outcome, we used the TriNetX's analytics function to perform propensity score matching and calculate the odds ratio (OR) for each outcome of interest. Regression analysess were not performed.

The data accessible through TriNetX as been attested to being de-identified through a formal determination by a qualified expert as defined in Section 164.514(b)(1) of the HIPAA Privacy Rule. Because of this determination, we did not seek, nor did we obtain, Institutional Board Approval for this research. TriNetX also offers built-in analytics, charts, graphs, and statistical options for data visualization. We stratified the patient cohort into three different age groups: Up to 6 years old, between 7 and 12 years, and between 13 and 18 years old. We also stratified the population based on weight by ICD codes normal weight (ICD 10: Z68.52), overweight (ICD 10: Z68.53), and obese (ICD 10: Z68.54). Furthermore, we created queries of patients who received influenza vaccination within one year of the outcome of interest (hospitalization, MIS-C diagnosis, or death), or COVID-19 vaccination, or both influenza vaccination and at least one COVID-19 vaccination before these outcomes. Visit codes were used for hospitalization/admission, ICD encounter diagnosis codes were used to identify MIS-C, and patient status codes were used to mortality.

Race categories included American Indian or Alaskan Native, Asian, African American/Black, Native Hawaiian or other Pacific Islander, White, and unknown. Ethnicity categories included Hispanic or Latino, Not Hispanic/Not Latino, and unknown. We used the TriNetX codes to identify the variables and outcomes of interest (see **Supplementary Material**). We used built-in analytic functions to calculate the incidence of COVID-19 among different age groups, the odds ratios of COVID-19 and MIS-C among the three weight groups, and the odds ratios of hospitalization, MIS-C diagnoses, and mortality for COVID-19 patients who were vaccinated against influenza, COVID-19, both, or neither. Chi-square tests were used to compare the observed incidence differences between genders, races, and ethnicities across different weight groups to the expected incidences. Multiplicative interaction was assessed for the outcomes of hospitalization, MIS-C, and mortality by dividing the odds ratio for COVID-19 infection for those who received both the influenza vaccine and the COVID-19 vaccine by the product of the odds ratio for the influenza vaccine only multiplied by the odds ratio for COVID-19 vaccine only. A ratio greater than 1.0 indicates positive interaction, whereas a ratio of less than 1.0 signifies negative interaction. An equal ratio of 1.0 represents no interaction on the multiplicative scale.

Results

Ethnicity and gender differences in the incidence rate of COVID-19 infection

The incidences of COVID-19 infection in (males/females) in the three age groups we investigated: Up to 6 years, between 7 and 12 years, and between 13 and 18 years old were

26.80/27.63%, 24.21/24.25%, and 23.63/23.09% respectively. The incidence of COVID-19 infection was higher among African American/Black race (28.57%) followed by White race (27.1%), patients with unknown/unreported race 21.22%, Native Hawaiian or other Pacific Islanders 20.92%, American Indian or Alaskan Native 19.92%, and Asian race 19.1%. In addition, the incidence of COVID-19 infection was 27.27% among patients of Hispanic ethnicity vs. 27.37% among non-Hispanic patients; patients with un-reported ethnicity had an incidence of 21.79% (Table 1).

Obesity increases the risk of developing COVID-19 infection in children of Native Hawaiian race and Hispanic or Latino ethnic background

The incidence of COVID-19 infection among normal weight, overweight, and obese patients were statistically different; 30.32%, 31.33%, and 31.91%, respectively, $p < 0.00001$. The distribution of all the ICD encounter diagnoses BMI categories in the general population (5th to <85th, 85th to <95th, and 95th or above) were similar to the distribution of the three BMI categories in the patients diagnosed with COVID-19 diagnoses among the same BMI categories (48.00(?)%, 18.00(?)%, 32.50(?)% vs. 47.59%, 18.89%, 33.52%). When analyzed using race, obesity increased the incidence of COVID-19 only among Native Hawaiian patients (45.13%). However, African American (38.26%), American Indian or Alaskan Natives (34.8%), and White (30.62%) patients, obesity did not increase the risk of COVID-19 infection. When analyzed by ethnic backgrounds, obesity diagnosis increased the risk of COVID-19 diagnosis among Hispanics/Latino population, 42.99% of but did not among Non-Hispanic/Non-Latino population 28.63% ($p < 0.00001$). A majority of patients that were Non-Hispanic or Non-Latino who developed COVID-19 infections had normal weight diagnoses (52.51%) as compared to the Hispanic and Latino ethnic backgrounds (36.24%). Most patients who have COVID-19 diagnosis and with unknown ethnicity (unreported), did not have an obesity diagnosis—45.16% had normal BMI, 19.91% were overweight, and 34.94% were obese. The results reflect that obesity as a suggested risk factor for COVID-19 infection should be interpreted in the context of racial and ethnic backgrounds (Table 2).

Increasing weight increases the risk of developing COVID-19 infection and MIS-C following COVID-19 infection in children

When adjusting for age, the incidence of COVID-19 was higher in patients with obesity compared to those with normal weight across all age groups (28.67% in patients up to 6 years old, 23.97% in patients between 7 and 12 years old, and 22.29% in patients between 13 and 18 years old) vs. (27.32%, 23.06%, and 21.54%), the odds ratios were 1.07 (95% CI 1.04–1.09), 1.05 (95% CI 1.03–1.07), and 1.04 (95% CI 1.03–1.06) respectively. The incidence of MIS-C was higher in total patients with an obesity diagnosis compared to patients with a normal weight diagnosis (0.041% vs. 0.024%, OR 1.71, CI 1.36,2.14), the higher incidence of MIS-C in obese patients was prominent across all age groups (0.057% in patients up to 6 years old, 0.047% in patients between 7 and 12-years old, and 0.03% in patients between13 and 18 years old) vs. (0.029%, 0.024%, and 0.02) respectively, the odds ratios were 1.95 (95% CI1.19–3.17), 1.98 (95% CI1.37–2.84), and 1.62 (95% CI1.11–2.36) respectively (Table 3).

Influenza vaccination, COVID-19 vaccination, either one alone or both significantly improve the outcomes in children with COVID-19 infection

Among COVID-unvaccinated patients, those who did not receive influenza vaccine within one year prior to COVID-19 infection had a higher incidence of hospitalization, MIS-C diagnosis, and death (8.4%, 0.08%, and 0.07%, respectively)

TABLE 1 Incidence of COVID-19 in pediatric population in different gender, race and ethnic background stratified by age group.

Age (years)		Up through 6	7–12	13–18	Total ≤18
Total number of populations (*n*)		4,683,222	5,993,104	6,482,320	17,158,646
COVID-19 patients (*n*) (%)		930,016 (27.16)	673,323 (24.16)	741,477 (23.31)	2,344,816 (24.97)
Gender with COVID-19 diagnosis	Female	427,773 (26.8)	322,458 (24.21)	382,603 (23.63)	1,132,798 (24.91)
	Male	502,010 (27.63)	350,687 (24.25)	358,565 (23.09)	1,211,262 (25.15)
	Unknown	269 (2.26)	178 (2.03)	309 (3.57)	756 (2.58)
Race with COVID-19 diagnosis	American Indian or Alaskan native	4,033 (21.83)	2,859 (19.17)	3,179 (18.53)	10,107 (19.92)
	Asian (*n*) (%)	21,666 (22.73)	14,507 (18.45)	12,482 (15.45)	48,655 (19.1)
	African American/Black	158,110 (33.05)	102,385 (26.84)	105,964 (25.06)	366,459 (28.57)
	Native Hawaiian or other Pacific Islander	953 (20.29)	805 (22.63)	874 (20.21)	2,632 (20.92)
	Unknown	293,086 (22.86)	199,045 (20.26)	210,061 (20.11)	702,192 (21.22)
	White	452,168 (29.25)	353,686 (26.68)	408,917 (25.39)	1,214,771 (27.1)
Ethnicity with COVID-19 diagnoses	Hispanic or Latino	150,727 (31.15)	96,252 (25.97)	95,775 (23.81)	342,754 (27.27)
	Not Hispanic/not Latino	443,992 (30.55)	325,441 (26.43)	354,782 (24.94)	1,124,215 (27.37)
	Unknown ethnicity	335,297 (22.54)	251,630 (21.24)	290,920 (21.46)	877,847 (21.79)

≤, less than or equal to; %, percent; *n*, number.

TABLE 2 Incidence of COVID-19 in pediatric population stratified by weight category.

Weight category		Normal weight *n* (percent)	Overweight *n* (percent)	Obese *n* (percent)
Number in the general population with weight category reported (*n* = 1,203,905)		586,341 (48%)	225,213 (18%)	392,351 (32.50%)
Incidence rate of COVID-19 diagnosis among patients (*n* = 373,536)		30.32%	31.33%	31.91%
Distribution of weight categories in patients with COVID-19 diagnosis (*n* = 373,536)		177,778 (47.59%)	70,559 (18.89%)	125,199 (33.52%)
Gender	Female (*n* = 162,745)	78,206 (48.05%)	32,460 (19.95%)	52,079 (32%)
	Male (*n* = 169,134)	81,400 (48.13%)	31,078 (18.38%)	56,656 (33.50%)
	Unknown (*n* = 45)	21 (46.67%)	10 (22.22%)	14 (31.11%)
Race	American Indian or Alaskan native (*n* = 1,618)	718 (44.37%)	337 (20.83%)	563 (34.8%)
	Asian (*n* = 8,874)	5,310 (59.84%)	1,623 (18.29%)	1,941 (21.87%)
	African American/black (*n* = 64,985)	26,830 (41.29%)	13,289 (20.45%)	24,866 (38.26%)
	Native Hawaiian or other pacific islander (*n* = 421)	149 (35.39%)	82 (19.48%)	190 (45.13%)
	Unknown (*n* = 67,774)	31,306 (46.19%)	12,928 (19.07%)	23,540 (34.37%)
	White (*n* = 188,249)	95,314 (50.63%)	35,286 (18.74%)	57,649 (30.62%)
Ethnicity	Hispanic or Latino (*n* = 60,440)	21,902 (36.24%)	12,553 (20.77%)	25,985 (42.99%)
	Not Hispanic/not Latino (*n* = 205,918)	108,119 (52.51%)	37,941 (18.43%)	59,858 (28.63%)
	Unknown ethnicity (*n* = 65,563)	29,606 (45.16%)	13,051 (19.91%)	22,906 (34.94%)

≤, less than or equal to, %, percent, *n*, number.

TABLE 3 Incidence and odd ratios of COVID-19 infection and multisystem inflammatory syndrome in children (MIS-C) stratified by weight category.

Weight category/age (y)		Up through 6	7–12	13–18	Total ≤18
COVID-19 infection	Normal weight (Reference group)				
	N of patients	146,232	218,692	221,296	586,220
	N of patients with the outcome	39,947	50,434	47,676	138,057
	Incidence %	27.32	23.06	21.54	23.55
	Overweight				
	N of patients	37,142	102,415	102,855	242,214
	N of patients with the outcome	10,396	23,853	22,839	57,088
	Incidence (%)	27.99	23.29	22.2	23.55
	OR (95% CI)	1.03 (1.00–1.06)	1.01 (0.99–1.03)	1.04 (1.02–1.06)	1.01 (1.00–1.02)
	P value	0.38	0.1	<0.0001	0.16
	Obese				
	N of patients	45,328	140,160	206,292	391,780
	N of patients with the outcome	12,998	33,591	45,992	92,581
	Incidence (%)	28.67	23.97	22.29	23.63
	OR (95% CI)	1.07 (1.04–1.09)[a]	1.05 (1.03–1.07)[a]	1.04 (1.03–1.06)[a]	1.003 (0.99–1.01)
	P value	<0.0001	<0.0001	<0.0001	0.105
MIS-C	Normal weight (Reference group)				
	N of patients	146,333	218,709	221,272	586,314
	N of patients with the outcome	43	52	45	140
	Incidence %	0.029	0.024	0.02	0.024
	Overweight				
	N of patients	37,176	102,417	102,849	242,442
	N of patients with the outcome	10	36	26	72
	Incidence (%)	0.027	0.035	0.025	0.03
	OR (95% CI)	0.91 (0.46–1.08)	1.48 (0.97–2.26)	1.24 (0.772.01)	1.25 (0.93–1.67)
	P value	0.96	0.11	0.25	0.32
	Obese				
	N of patients	45,429	140,501	206,273	392,203
	N of patients with the outcome	26	66	68	160
	Incidence (%)	0.057	0.047	0.03	0.041
	OR (95% CI)	1.95 (1.19–3.17)[a]	1.98 (1.37–2.84)[a]	1.62 (1.11–2.36)[a]	1.71 (1.36–2.14)[a]
	P value	0.005	0.001	0.002	0.004

≤, less than or equal to; <, less than; %, percent; *n*, number; MIS-C, multisystem inflammatory syndrome in children.
[a]indicates statistically significant.

compared to those who were influenza-vaccinated (7.14%, 0.06%, and 0.05%), the odds ratio and confidence interval are (OR 1.19, 95% CI 1.1–1.28), (OR 1.52, 95% CI 1.32–1.74), and (OR 1.47, 95% CI 1.26–1.71) respectively (Table 4).

In the same context, among influenza-unvaccinated patients who did not receive COVID-19 vaccine before their COVID-19 diagnosis had higher incidence of hospitalization (10.55%) and development of MIS-C (0.09%) compared to those who received at least one dose of COVID-19 vaccine before their COVID-19 infection (9.56% vs. 0.10%) respectively, the odds ratio and confidence interval are (OR 1.11, 95% CI 1.04–1.19) and (OR 0.84, 95% CI 0.45–1.57) respectively. Similarly, the mortalities were higher (0.11% among COVID-19-unvaccinated compared to 0.10% among COVID-19-vaccinated), the odds ratio and confidence interval are (OR 1.12, 95% CI 0.6–2.08) respectively (Table 4). The incidence of hospitalization (8.4%) and MIS-C diagnosis (0.08%) was higher among patients who were not vaccinated against neither Influenza nor COVID-19 within one year prior to COVID-19 infection compared to patients who were vaccinated against both for Influenza and COVID-19 (5.91% and 0.02% respectively) (OR 1.46, CI 1.41–1.51 and OR 3.72, CI 2.11–6.56 respectively). Similarly, the mortality rate was also found higher in the unvaccinated group (0.07%) as compared to the group vaccinated against both infection (0.02%) (OR 13.55, CI 1.91–9.62). (Table 4). Using the multiplicative interaction calculation, we found a positive interaction between the COVID-19 vaccine and influenza vaccine for all outcomes. While the interaction is modest for hospitalization after COVID-19 infection (ratio = 1.1), the interaction was strong for MIS-C (ratio = 2.9) and mortality (ratio = 2.2) outcomes, indicating that the presence of both vaccinations had a stronger impact than what would be expected by the combination of each vaccine's independent effect.

Discussion

To understand the true burden of COVID-19, it is critical to consider patient characteristics and their consequences on the public health response. Our study analyzed a global cohort of 17,158,646 pediatric patients, the most extensive data set reported thus far. The key results of our study are: (1) there are no gender differences in the incidence of COVID-19 infection in children, (2) there are no ethnic (Hispanic or Latino/Not Hispanic or Not Latino) differences in the incidence of COVID-19 infection in children, (3) Race (American Indian or Alaskan Native, Asian, African American/Black, Native Hawaiian or other Pacific Islander, White, and unknown) differences are similar to those reported from small cohorts previously with African American/Black patients being at highest risk, (4) COVID-19 infection is more common in younger age groups (up to 6 years old), (5) COVID-19 patients of Native Hawaiian race, and those of Hispanic ethnicity are more likely to be obese, (6) the odds of MIS-C were higher in patients with at least one ICD encounter diagnosis code for overweight or obesity, as compared to patients with a normal weight ICD encounter diagnosis code, (7) patients who were vaccinated against influenza alone, or COVID-19 alone, or vaccinated against both have better

TABLE 4 The odds of hospitalization, MIS-C, and mortality among pediatrics patients according to their COVID-19 /influenza vaccination status.

Unvaccinated against influenza or COVID-19 (*n*)	1,921,341	Incidence (%)	OR CI (95%) *P* value	Unvaccinated against influenza or COVID-19 (*n*)	1,921,341	Incidence (%)	OR CI (95%) *P* value	Unvaccinated against influenza or COVID-19 (*n*)	1,921,341	Incidence (%)	OR CI (95%) *P* value
Influenza vaccinated (*n*)	**422,683**			**Covid-19 vaccinated (*n*)**	**9,824**			**Double vaccinated**	**52,872**		
Unvaccinated	Hospitalization (n)	161,318	8.4	1.19 (1.18–1.21) <0.0001	Unvaccinated	Hospitalization (n)	208,025	10.82	1.11 (1.04–1.19) 0.002	Unvaccinated	Hospitalization (n)	161,318	8.4	1.46 (1.41–1.51) <0.0001
Influenza-vaccinated	Hospitalization (n)	30,192	7.14		Covid vaccinated	Hospitalization (n)	943	9.56		Double vaccinated	Hospitalization (n)	3,127	5.91	
Unvaccinated	MIS-C (n)	1,637	0.08	1.52 (1.32–1.74) <0.0001	Unvaccinated	MIS-	1,691	0.09	0.84 (0.45–1.57) 0.59	Unvaccinated	MIS-C	1,637	0.08	3.72 (2.11–6.56) <0.0001
Influenza vaccinated	MIS-C (n)	235	0.06		Covid vaccinated	MIS-C (n)	10	0.10		Double vaccinated	MIS-C (n)	12	0.02	
Unvaccinated	Mortality (n)	1,290	0.07	1.47 (1.26–1.71) <0.0001	Unvaccinated	Mortality (n)	2,242	0.12	1.12 (0.60–2.08) 0.73	Unvaccinated	Mortality (n)	1,290	0.07	3.55 (1.91–9.62) <0.0001
Influenza vaccinated	Mortality (n)	193	0.05		Covid vaccinated	Mortality (n)	10	0.10		Double vaccinated	Mortality (n)	10	0.02	

Unvaccinated = neither received Influenza vaccine (within 1 year before the outcome), nor received Covid-19 vaccine before COVID-19 diagnoses. Influenza vaccinated = received Influenza vaccine (within 1 year before the outcome) but not Covid-19 vaccine. Covid-19 vaccinated = received Covid-19 vaccine before the outcome but not Influenza vaccine within 1 year before the outcome. Double vaccinated = received both Influenza vaccine (within 1 year before the outcome), and at least one dose of Covid-19 vaccine before the COVID-19 diagnosis. *n*, number; %, percent; <, less than; OR, odds ratio; CI, confidence interval; MIS-C, multisystem inflammatory syndrome in children.

outcomes for hospitalization, MIS-C, and mortality compared to patients who are not vaccinated against either Influenza, or COVID-19, or both. Most importantly, we show that having received both COVID-19 and influenza vaccination have a multiplicative protective effect than each separately.

Our study is the largest of its kind (to date) targeting to explore the global demographics of the pandemic using TriNetX network, and the first of its kind analyzing the impact of influenza vaccination alone, COVID-19 vaccination alone, or both vaccination in COVID-19-related disease severity such as hospitalization, MIS-C diagnosis, or mortality among pediatric patients.

Unlike other coronaviruses, SAR-CoV-2 has been recognized to cause a unique post-viral immune reaction, a multisystem inflammatory syndrome in children (MIS-C) (21, 22). MIS-C presents as a critical febrile illness, and patients might be hospitalized in intensive care units and receive respiratory support for weeks. MIS-C has been suggested to significantly increase the risk of death in children, unlike acute infection. One of the theories behind the etiopathogenesis of MIS-C, which was not described in the previous coronavirus outbreaks, is the possibility of a residual viral infection that the host is developing a hypersensitivity reaction against (26). Children are found to shed viral particles in respiratory secretions and stool during acute infection, through the convalescent period and beyond (10–13). Viral particles were found in the respiratory secretions and stool of MIS-C patients (13). Our results indicate that MIS-C by itself is seen less often in vaccinated children. Even a previous vaccination with influenza vaccine seems to help protect children from developing MIS-C, an observation which is supported by evolving literature; Domnich et al., in an observational study found a protective effect of influenza vaccine against COVID-19 infection (16). Similarly, a recent meta-analysis reported lower risk of SARS-CoV-2 infection among influenza vaccinated participants (27), the protective effect is likely related to the non-specific activation of innate immunity after influenza vaccination which would enhance the production of proinflammatory cytokine, triggering a non-specific protection against other viruses (28).

Previous reports highlighted the need to untangle the SARS-CoV-2 immunologic response differences in race/ethnicity based on the claim that Covid-19 infection is likely related to socioeconomic status, not race nor ethnicity (14, 15). A recent study reported that there are higher infection and mortality rates in counties with higher poverty rates and with more, Non-White, and more non diverse populations (17). In this project, we used global data from TriNetX; we did not assess the COVID-19 infection risks of patients in different socioeconomic backgrounds. However, the TriNetX cohort we explored reveals a higher incidence of COVID-19 infection among patients of African American/Black race. Because ethnicity was a separate variable and can be assessed separately from the "race" variable, we analyzed the data using the ethnicity variable as well. Surprisingly, we found no ethnicity differences (Hispanics vs. non-Hispanics) in the incidence of COVID-19 infection in children. Our study did not find significant gender differences in the incidence of COVID-19 infections. We found gender and ethnicity effects in COVID-19 infection incidence rates when stratified by weight categories.

Data on the relationship between COVID-19 infection and obesity in the pediatric population is scarce. Our study suggests that the overall incidences of COVID-19 infection increased statistically with increasing weight category—normal weight, overweight, and obese—but this finding is probably clinically insignificant because of our large sample size. However, obesity as a suggested risk factor for COVID-19 infection should be interpreted in the context of race and ethnicity, as we noticed a higher proportion of patients with COVID-19 of Native Hawaiian race, and of Hispanic ethnicity have and overweight or obesity diagnosis. Our findings are similar to a nationwide case-control study from South Korea, which included 3,788 COVID-19 cases and 15,152 matched controls; even after adjustment for sociodemographic, comorbidity, laboratory, and medication data, higher weight category levels were associated with a higher risk of contracting COVID-19 infections (18). However, this study did not report the race of the participant (18). It is anticipated that most of the patients in the South Korean cohort were Asian, and the number of participants is small compared to our study.

Our finding of a higher incidence of MIS-C among patients with higher BMI supports the previously published studies that obesity is associated with increased COVID-19 severity (5, 19–22). In addition, a study on 494 COVID-19-infected children suggested that the association between obesity and COVID-19 severity is age-dependent and that obesity increases the risk for COVID-19-related critical illness in adolescents but not in younger children (8). However, our findings do not support these findings. Interestingly, the same study reported that higher BMI was not associated with length of hospitalization, ICU admission, myocarditis, MIS-C, acute kidney injury, or mortality for any age (8). In our study, we only analyzed MIS-C as a sequela of COVID-19 infection and did not study the post-infectious single system involvement such as cardiac or renal systems.

To date, there are no pediatric reports about the association between prior Influenza vaccination, or both Influenza vaccination and COVID-19 vaccination, and the outcomes of COVID-19 infection. However, data from adult studies suggested that influenza vaccination might have a protective effect against COVID-19 infection and reports less severe acute illness presentation in previously influenza vaccinated adults (23–25). Our data uniquely demonstrate that influenza vaccination, COVID-19 vaccination, or both significantly improve the outcomes in children with COVID-19 infection. Using the multiplicative interaction scale, we found a positive interaction between the COVID-19 vaccine and influenza vaccine; they both combined have a more significant effect than each separately; the positive interaction is modest for hospitalization risk; however, the interaction was strong for MIS-C and mortality risk.

Our study has several limitations. First of all, our study has a potential bias having about 20%–22% of COVID-19 infected patients with unknown race or ethnicity, which possibly skewed the results towards higher incidence in one race or ethnicity and underestimated the incidence in other races or ethnicities, however giving the magnitude of the cohort, the potential of this skew is unlikely. Using the TriNetX system, we think that our analysis is limited due to building the cohort query based on supported code systems only. It is possible that many patients were missed if the

correct code was not used during patient care. Another limitation of our study is that our methods do not let us identify the exact reason for hospitalization post COVID-19 infection. We used the ICD 10-codes to identify patients with normal weight, overweight, and obesity (n = 1,203,905). We did not use the actual weight or BMI of each patient. Therefore, our data did not include 90% of the total patients in the cohort (17,158,646). Nevertheless, the sample size of 1,203,905 patients with weight category, based on ICD encounter diagnoses, reported is large enough to draw an association between COVID-19 infection, race, and ethnicity, through the weight categories, and an association between weight categories and MIS-C risk. Another limitation of our work is that our cohorts were built based on a single dataset, which is approximately 64% from US, and approximately 23% from European, Middle Eastern and African countries all together. However, the dataset contained EHR data from over 100 million patients.

Conclusion

Scientists have reached a considerable level of understanding of COVID-19 infection in children and are starting to understand MIS-C better. Further studies are needed to better understand the relationship between obesity and pediatric severe COVID-19 infection and post-infection sequela, including MIS-C. Our work displayed the synergistic effect of being vaccinated against both COVID-19 and influenza, which encourage researchers to develop a combined vaccine in the future. Future studies examining the immunologic responses of individuals to COVID-19 infection who were recently vaccinated by other viral infection vaccines are needed. Nevertheless, more extensive, and collaborative prospective studies are needed to understand the relation between COVID-19 illness and influenza vaccination and the potential relation between COVID-19 host defense and other vaccines, which could explain why the pediatric population has less severe COVID-19 infection and infection-related outcomes as compared to adults in general.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1046680/full#supplementary-material

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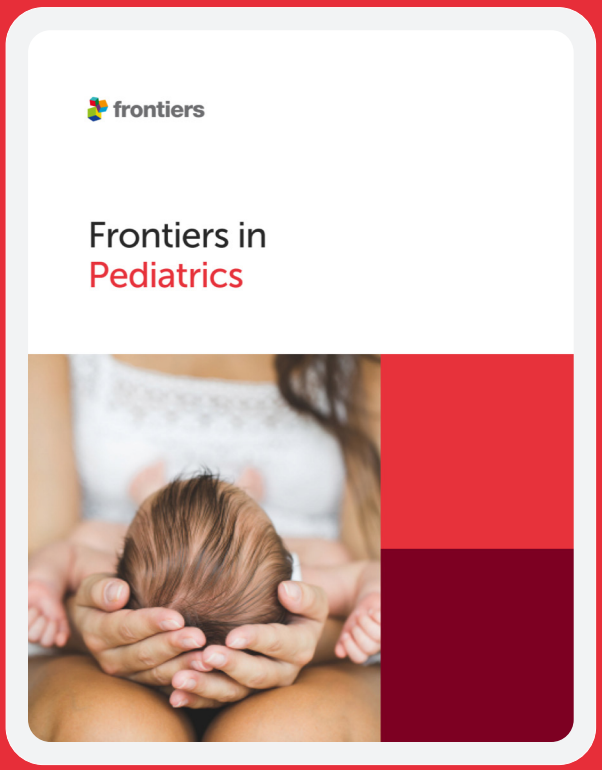

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