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# Exhaled Breath Condensate and Respiratory Sequelae in Children Post-COVID-19

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**Keywords**
Exhaled breath condensate · Lung function · Functional capacity · Pediatric respiratory sequelae of severe acute respiratory syndrome coronavirus 2 infection

**Abstract**
***Background:*** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes an acute respiratory illness. A substantial proportion of adults experience persistent symptoms. There is a paucity of data on respiratory sequelae in children. Exhaled breath condensate (EBC) is a non-invasive tool used to assess airway inflammation. ***Objectives:*** This study aimed to evaluate EBC parameters, respiratory, mental and physical ability among children post COVID-19 infection. ***Methods:*** Observational study of confirmed SARS-CoV-2 infection cases among children, aged 5–18 years, evaluated once, 1–6 months post positive SARS-CoV-2 PCR testing. All subjects performed spirometry, 6-min walk test (6MWT), EBC (pH, interleukin-6), and completed medical history questionnaires, Depression, Anxiety, and Stress Scale (DASS-21), and physical activity scores. Severity of COVID-19 disease was classified according to WHO criteria. ***Results:*** Fifty-eight children were included and classified asymptomatic ($n$ = 14), mild ($n$ = 37), and moderate ($n$ = 7) disease. The asymptomatic group included younger patients compared to the mild and moderate groups (8.9 ± 2.5y vs. 12.3 ± 3.6y and 14.6 ± 2.5y, respectively, $p$ = 0.001), as well as lower DASS-21 total scores (3.4 ± 4 vs. 8.7 ± 9.4 and 8.7 ± 0.6 respectively, $p$ = 0.056), with higher scores in proximity to positive PCR ($p$ = 0.011). No differences were found between the 3 groups regarding EBC, 6MWT, spirometry, body mass index percentile, and activity scores. ***Conclusions:*** COVID-19 is an asymptomatic-mild disease in most young healthy children, with gradually diminishing emotional symptoms. Children without prolonged respiratory symptoms revealed no significant pulmonary sequelae as evaluated by EBC markers, spirometry, 6MWT, and activity scores. Larger studies are required to assess long-term pediatric consequences of post SARS-CoV-2 infection, to assess the need for pulmonology surveillance.


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## Introduction

Acute coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, typically presents as a respiratory illness. Children often show milder disease and better prognosis than adults [1]. The long-term follow-up of adults shows that a large proportion continue to experience fatigue and dyspnea months after diagnosis [2, 3]. Pulmonary function test can be unaffected, but there are reports of decreased diffusion capacity for carbon monoxide (DLCO), 6-min walk test (6MWT), and interstitial lung abnormalities post severe acute COVID-19 [3–5]. Initial reports on persisting symptoms in children with long COVID have been published [6–8], but there is a lack of objective evaluations and data on post-acute respiratory sequelae of SARS-CoV-2 infection in children without prolonged symptoms.

Long COVID is a multisystem condition that develops regardless of the initial disease severity. The mechanisms underlying its pathophysiology remain unclear. A number of long-lasting inflammatory mechanisms have been proposed [9]. However, psychological factors can also contribute to the development of post-COVID-19 fatigue [10]. According to the "biopsychosocial model," a person's medical condition is not only simply determined by the biological but also by psychological and social factors. Physical and mental fatigue are major symptoms of long-COVID syndrome. This often leads to depression and anxiety symptoms, causing even more fatigue [11]. Thus, assessment of long-COVID sequels should include both psychological and biological factors. In pediatrics, the potential for non-invasive assessment of airway inflammation, pulmonary exacerbation, and the severity of lung disease is of paramount importance [12–14]. Exhaled breath condensate (EBC) is a simple non-invasive tool, which can be used to assess airway inflammation. Specifically, pH of EBC is a reliable and replicable measurement, with a "normal range" confirmed in healthy adults [15]. Airway acidification was demonstrated in several chronic lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, chronic cough, and bronchiectasis [16]. Another important indicator for evaluating COVID-19 effects could be the level of interleukin (IL)-6 in EBC, due to its role in the systemic hyper-inflammatory response induced by SARS-CoV-2 [17].

Additional domains of the effects of COVID-19 are the emotional [18] and physical [8] capacities in children post-acute COVID-19. Simple measurements can be conducted using 6MWT, exercise, and mental health questionnaires.

Considering that the COVID-19 pandemic affects a large number of children around the globe, it is vital to understand the scope and severity of respiratory sequelae among recovered COVID-19 pediatric patients. The aim of the current study was to evaluate EBC parameters, lung function, mental and physical ability in a cohort of recovered COVID -19 infection pediatric patients.

## Materials and Methods

### *Study Design and Participants*

The study was a prospective, single center, observational study of children between age 5 and 18 years who were able to perform spirometry and EBC collection. SARS-CoV-2 infection was microbiologically confirmed by real-time quantitative reverse transcription polymerase chain reaction (PCR) during acute infection which was 1–6 months prior to study evaluation. Children with diagnosis of chronic lung diseases (such as current asthma, congenital airway malformations, cystic fibrosis, bronchopulmonary dysplasia); upper respiratory tract infection in the previous 2 weeks; current or past smoking; or systemic inflammatory diseases (such as inflammatory bowel disease, systemic lupus erythematosus, sarcoidosis) were excluded. Subjects did not use any inhaled medications or oral corticosteroids regularly or within 2 weeks prior to the study visit.

Children with confirmed positive COVID-19 PCR between September 2020 and April 2021 were recruited using Israel's largest health care organization's database (Clalit Health Services, Haifa, and Western Galilee district). The participants were invited to a one-visit assessment at the Pediatric Pulmonology Unit at Carmel Medical Center, Haifa, Israel from March through June 2021. The institutional review board approved the study protocol (IRB approval number 0008-21-CMC). A parent provided written informed consent prior to any study assessment. During the visit, a review of the medical record including data on acute COVID-19 disease and physical examination were performed to meet inclusion and exclusion criteria. Participants then performed spirometry, 6MWT, EBC, and completed three questionnaires: (1) demographic and past medical history including symptoms related to asthma and allergy; (2) DASS-21; (3) the Godin Leisure-Time Exercise Questionnaire. Severity of the acute COVID-19 disease was classified according to the World Health Organization (WHO) symptom severity criteria [19]. The research definition for "long COVID" (synonymous with "post-COVID-19 condition") in children and young people, aligned to the clinical case definition of the WHO [20], was proposed by Delphi consensus process as follows: post-COVID-19 condition occurs in young people with a history of confirmed SARS- CoV-2 infection, with at least one persisting physical symptom for a minimum duration of 12 weeks after initial testing that cannot be explained by an alternative diagnosis. The symptoms have an impact on everyday functioning, may continue or develop after COVID infection, and may fluctuate or relapse over time. [21].

*Study Assessments*

EBC Sample Collection

We used a portable condenser (transportable unit for research on biomarkers obtained from disposable exhaled condensate collection systems; TURBODECCS; ItalChill, Parma, Italy), which is specifically designed to collect EBC in clinical settings. The use of TURBODECCS in this context has been validated [22]. There was no alveolar air valve in the turbo condenser used, thus the alveolar and bronchial air fractions were collected together. The condenser has a refrigerating system (TURBO) that thermostatically controls the working temperature, and a disposable respiratory system (DECCS) that consists of a mouthpiece connected to a one-way aspiration valve and an EBC collection test tube at the end. Subjects were asked to perform normal tidal breathing into the mouthpiece for 10 min at an initial condenser temperature of −5°C to collect 1–2 mL samples.

Due to the inclusion of preschoolers who are incapable of performing tests with nasal clips, we did not use a nasal clip in this study. Subjects were asked to avoid eating 2–3 h prior to EBC collection. Since past and current smokers as well as subjects who use inhaled medication were excluded, no specific recommendations were given in regards to smoking or inhaled medication. All EBC samples were collected between 10 a.m. and 18 p.m. in an environment with room temperature (22–23°C) and humidity (50%) controlled by a closed air-conditioning system.

IL-6 and pH measurements in EBC were the primary endpoints. The pH was measured using a pH meter (Hanna Instruments, Leighton, UK) after argon gas was passed over the sample at 2 L/min for 10 min with a sensitive electrode. pH measurement was performed twice, before and after CO2 deaeration (Hamilton, Reno, NV). IL-6 was measured using a Cobas 6000 analyzer (Roche Diagnostics).

Secondary endpoints included pulmonary and functional evaluations: (1) spirometry measures including percent predicted forced expiratory volume in 1s (FEV1), forced vital capacity (FVC), and forced expiratory flow between 25 and 75% of the vital capacity using Global Lung Function Initiative (GLI) reference data [23]. Lower limit of normal was defined as Z scores below 1.64. Spirometry was performed in accordance with standardization [24] using a KoKo® spirometer. (2) 6MWT was performed in accordance with guidelines with pre and post vital signs. The 6MWT is a simple, safe, easy to administer, well-tolerated, and well-established submaximal exercise test used to quantify the functional exercise capacity in children and adults. The primary measurement is 6-min walk distance (6MWD) in meters [25]. (3) Godin Leisure-Time Exercise Questionnaire is a self-report validated questionnaire which assesses frequency, duration, and intensity of physical activity during a typical week in the patient's life. Using a standard equation, each patient receives total leisure activity score that provides further information of his physical functional capacity [26].

We also assessed emotional parameters with the Depression, Anxiety, and Stress Scale (DASS-21) [27] which is a short self-report validated questionnaire that includes 7 questions in each domain assessing the previous week in the patient's life. It is used to measure the dimensions of depression, anxiety, and stress, and sums up to a total scale – with higher score indicating a higher emotional impact.

*Statistical Analysis*

Statistical analysis was performed using IBM statistics SPSS version 24. The continuous variables were presented by mean and standard deviation and median and interquartile range. The categorical variables were presented in percentages. Differences in demographics and clinical characteristics between the three groups (asymptomatic, mild, moderate) were analyzed using the Kruskal-Wallis test followed by Mann-Whitney, for the continuous variables. $\chi^2$ with Bonferroni correction for pairwise comparisons was used for comparing the categorical variables. $p < 0.05$ was considered statistically significant.

## Results

Six hundred and twelve out of 1,180 children, aged 5–18 years, with confirmed positive COVID-19 PCR were contacted, of them 58 children were included in the study (Fig. 1). They were classified retrospectively as: asymptomatic ($n$ = 14), mild ($n$ = 37), and moderate ($n$ = 7) acute COVID-19. Fifteen percent ($n$ = 9) of the children reported long-COVID-related symptoms at the time they arrived for evaluation. The most common symptoms were as follows: dyspnea ($n$ = 4), fatigue ($n$ = 3), chest pain ($n$ = 2), back pain ($n$ = 2), headache ($n$ = 1), and anosmia ($n$ = 1) (online suppl. Table. 1; for all online suppl. material, see https://doi.org/10.1159/000530971). All but one of these patients was symptomatic during the acute illness. There were no significant differences in the demographic and clinical characteristics between the groups (Table 1), except for mean age. The asymptomatic group included younger patients in comparison to the mild and moderate groups (8.9 ± 2.5y vs. 12.3 ± 3.6y and 14.6 ± 2.5y, respectively, $p$ = 0.001). The mean body mass index (BMI) percentile by Center for Disease Control and Prevention (CDC) growth charts for age in our sample was 63.6 ± 27.3; there were 7 (12%) overweight (BMI between 85th and 95th percentile) and 9 (15%) obese (BMI >95th percentile). Regarding respiratory and atopic childhood manifestations (Table 2), there was a trend toward increased disease severity classification in children with a history of symptoms in exertion (asymptomatic 0, mild 5%, moderate 29%, $p$ = 0.05), wheezing episode in the last year (asymptomatic 0, mild 0, moderate 29%, $p$ = 0.013), and past inhaled corticosteroids use (asymptomatic 0, mild 22%, moderate 29%, $p$ = 0.06). Regarding emotional impact, DASS-21 total scale was elevated in mild and moderate groups compared to the asymptomatic group (8.7 ± 9.4 and 8.7 ± 0.6 vs. 3.4 ± 4, respectively, $p$ = 0.056). All the above were non-significant after sample size adjusting (Bonferroni correction), except for mean age. We found no differences among the 3 groups regarding pH and IL-6 EBC levels, 6MWT, spirometry indices, and total leisure activity score 1–6 months' post COVID-19 (Table 3).

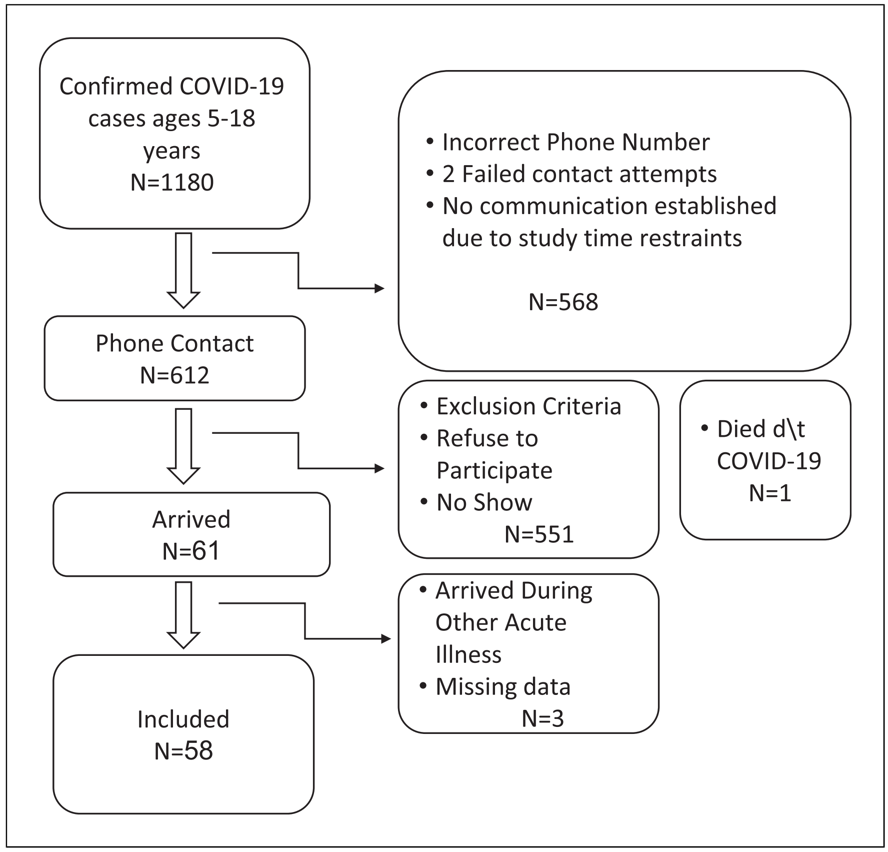


**Fig. 1.** Cohort study flowchart.



The results of parameters presented in Table 3 did not change by grouping patients according to time from positive PCR result (1–2 m vs. 2–4 m vs. 4–6 m), except for the DASS-21 which was higher between 1 and 2 m (6 patients: asymptomatic 1, mild 4, moderate 1; mean 15.7 ± 7.8) as opposed to 4–6 m (20 patients: asymptomatic 4, mild 13, moderate 3; mean 5.8 ± 5.9), $p = 0.011$ after Bonferroni correction.

## Discussion

This prospective preliminary study provides insight to the inflammatory, respiratory, and functional consequences of healthy children post COVID-19. Acute COVID-19 is an asymptomatic-mild disease in most young children as reported in the literature [28], with moderate severity with increasing age, but without severe or critical representation in this study.

It is important to note that most children (85%) in this study did not suffer from post-acute or long-COVID respiratory symptoms and did not have functional impairment in their daily living, which probably reflects the non-significant respiratory and physical ability sequelae as shown in the spirometry, 6MWT, and physical activity scores which did not differ among groups. This observation needs to be validated in large-scale research, in order to evaluate the importance of surveillance for respiratory impairment in children post-COVID-19. This is in contrast to a study which evaluated a different cohort than ours [8] mainly previously healthy children with persistent symptoms who presented to a designated multidisciplinary clinic for long-COVID evaluation. Five (8.3%) of 60 patients who underwent spirometry due to cardiorespiratory symptoms showed mild obstructive patterns, 15/55 showed air trapping by plethysmography, and 15/29 exhibited reversibility of the obstructive defect [8]. Another recent study [29] in children with protracted respiratory symptoms, demonstrated normal spirometry and plethysmography in most patients as in our study, but the 6MWT revealed exercise intolerance and significant tachycardia in 6/9 children tested.

There was no significant difference in BMI percentile between the three study groups; however, it is important to note that the present cohort included 27% overweight children. A high prevalence of obesity is observed in

**Table 1.** Demographic and clinical characteristics of study population ($n = 58$)

| | Asymptomatic (*n* = 14) | Mild (*n* = 37) | Moderate (*n* = 7) | *p* value |
|---|---|---|---|---|
| Age, years (mean±SD) | 8.9±2.5 | 12.3±3.6 | 14.6±2.5 | **0.001** |
| Male, *n* (%) | 4 (29) | 21 (57) | 4 (57) | 0.24 |
| BMI percentile (mean±SD) | 65.3±24.9 | 64.9±26.8 | 54.5±34.9 | 0.67 |
| Gestational week (mean±SD) | 39.9±1.1 | 38.6±2.4 | 38.9±1.9 | 0.46 |
| Smoking exposure, *n* (%) | 5 (36) | 3 (8) | 0 (0) | 0.25 |
| Socioeconomic status Mod-high, *n* (%) | 13 (93) | 36 (97) | 7 (100) | 0.97 |

BMI, body mass index.

**Table 2.** Respiratory and atopic childhood manifestations of study population ($n = 58$)

| | Asymptomatic (*n* = 14) | Mild (*n* = 37) | Moderate (*n* = 7) | *p* value |
|---|---|---|---|---|
| Family history of asthma, *n* (%) | 3 (21) | 13 (35) | 3 (43) | 0.65 |
| Diagnosis of asthma in the past, *n* (%) | 0 (0) | 2 (5) | 1 (14) | 0.33 |
| Atopic dermatitis, *n* (%) | 1 (7) | 3 (8) | 1 (14) | 0.99 |
| Allergy, *n* (%) | 1 (7) | 7 (19) | 1 (14) | 0.68 |
| Positive prick test, *n* (%) | 0 (0) | 4 (11) | 1 (14) | 0.38 |
| Past ICS use, *n* (%) | 0 (0) | 8 (22) | 2 (29) | **0.06** |
| Ever wheezing, *n* (%) | 1 (7) | 9 (24) | 2 (29) | 0.4 |
| Wheezing episode in last 12 months, *n* (%) | 0 (0) | 0 (0) | 2 (29) | **0.013** |
| Past exertional cough, *n* (%) | 0 (0) | 2 (5) | 2 (29) | **0.05** |

ICS, inhaled corticosteroid.

**Table 3.** Post-COVID emotional, functional, and inflammatory parameters of study population ($n = 58$)

| Mean±SD | Asymptomatic (*n* = 14) | Mild (*n* = 37) | Moderate (*n* = 7) | *p* value |
|---|---|---|---|---|
| DASS21 | 3.4±4 | 8.7±9.4 | 8.7±0.6 | **0.056** |
| Godin Leisure | 33±22 | 44±33 | 47±31 | 0.49 |
| 6MWT, m | 447±39 | 444±54 | 451±66 | 0.72 |
| Z score | 0.02±0.75 | 0.03±1.05 | 0.10±1.27 | |
| FEV1% | 101±15 | 99±9 | 97±11 | 0.76 |
| Z score | 0.11±1.37 | 0.0±0.84 | −0.23±1.01 | |
| FVC% | 100±15 | 98±11 | 96±12 | 0.81 |
| Z score | 0.12±1.20 | −0.0±0.88 | −0.21±1.23 | |
| FEF 25–75% | 94±23 | 98±21 | 96±14 | 0.57 |
| Z score | −0.13±1.10 | 0.06±1.02 | −0.06±0.68 | |
| EBC-pH | 7.5±0.75 | 7.6±0.55 | 7.7±0.1 | 0.44 |
| EBC-IL-6 (ng/mL) | 3±1.8 | 3.6±5.2 | 2.7±1.1 | 0.9 |

DASS21, Depression Anxiety and Stress Scale; Godin Leisure, Godin Leisure-Time Exercise Questionnaire; 6MWT, 6-min walk test; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF 25-75, forced expiratory flow between 25 and 75% of the vital capacity; EBC, exhaled breath condensate.

severe cases of COVID-19 in children and adolescents [30]. These results are similar to two studies that evaluated pediatric populations that presented with prolonged post-SARS-CoV-2 infection respiratory symptoms, which found an increased prevalence of obesity (62% [29] and 25.6% [8] with BMI percentile >85). Whether obesity is also linked to persistent symptoms after COVID-19 remains to be determined.

We did not find significant differences in EBC pH and IL-6 levels among the three study groups 1–6 months' post COVID-19. To the best of our knowledge, this is the first study to examine EBC specimens in a pediatric population post-acute COVID-19. A recent cross-sectional analysis of EBC-pH from 613 children aged 7–12 years, revealed an association between asthma and acidic pH (<7.5) [28]. In another study, lower pH-EBC levels were correlated with neutrophilic inflammation in various airway diseases under stable conditions [31]. However, some of these findings have not been replicated in large cohorts, despite plausible pathophysiological explanations for both children and adults [32]. Regarding IL-6, a recent review concluded that serum levels of IL-6 are significantly elevated in severe cases of COVID-19 and are associated with adverse clinical outcomes, including admission to intensive care units, acute respiratory distress syndrome, and death [33]. Our cohort did not include severe cases of COVID-19, which could explain our results. As this study is the first to evaluate EBC in post COVID subjects, it raises the question whether it is possible to demonstrate EBC inflammatory markers after SARS-CoV-2 infection and whether EBC parameters were higher during and in close proximity to the acute illness.

Regarding emotional impact, DASS-21 total scale was elevated in mild and moderate groups compared to the asymptomatic, and the mean score was also higher, closer to the acute COVID-19 disease than 4–6 months following the illness ($p$ = 0.011). Compounded by the psychological toll of the pandemic experienced throughout the general population, individuals recovering from COVID-19 may be at even greater risk of depression, anxiety, and post-traumatic stress disorder. Post-acute hospitalized COVID-19 patients who were followed up at 6 months reported anxiety, depression, and sleep difficulties [5]. An analysis of 62,354 recovered COVID-19 patients revealed that anxiety disorders had the highest hazard ratio, with an incidence of 18.1% between 14 and 90 days after diagnosis [34]. In the pediatric population, the overall prevalence of emotional and behavioral problems among children in China was 12.5% during the COVID-19 post-pandemic [35]. Another study from Italy [36] showed that parents whose children tested positive for COVID-19 were more prone to developing post-traumatic stress, anxiety, and depressive symptoms. The emotional problems of the caregivers can partially explain the psychological health of their children [36]. A different explanation for the long-term neuropsychiatric symptoms of COVID-19 (including depression and anxiety) is that IL-6 is a potential mediator of immunological alternation and inflammatory process which leads to these symptoms [37]. In this study, however, we found no correlation between disease severity and higher IL-6 levels in EBC.

This study suggests that respiratory but not atopic childhood background can lead toward increased acute COVID-19 disease severity classification, though we did not include active asthma patients in our cohort. These relations were non-significant after sample size adjustment. Larger studies are warranted to confirm these associations.

The study is limited by the small sample size and single-center design; also, a baseline pre-COVID evaluation of the patients is lacking. We did not perform diffusion capacity for carbon monoxide or plethysmography, but due to the normal results of spirometry and 6MWT, we wonder if it would have added information. In addition, alveolar and bronchial air fraction was not separated in EBC sampling. We believe however that measuring the total fraction EBC is also valuable, since studies have demonstrated both airway and alveolar long-COVID inflammatory sequels [38, 39].

Another limitation is that neither did we include severe and critical cases of acute COVID-19 disease, due to their rarity in children, nor did we include ancillary comorbidities. Further larger studies with all severity classes of COVID-19 are required to assess the short- and long-term pediatric respiratory consequences post SARS-CoV-2 infection.

## Conclusions

The burden of caring for individuals recovering from COVID-19 is expected to be substantial, and future medical and social interventions must consider the late sequelae of SARS-CoV-2 infection. Even though children in general experience less severe COVID-19 symptomatology than adults, our study demonstrated that children can have long-term emotional symptoms which may impact their quality of life. Our study indicates that in healthy children without prolonged respiratory complaints, respiratory and physical ability post COVID-19 are probably not affected. There will be an increasing need to follow these children longitudinally to determine the long-term consequences of SARS-CoV-2 infection on respiratory and mental health, as this information will have important implications for public health surveillance, health resource allocation, clinical research, and future treatments.

## Statement of Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The institutional board of Carmel Medical Center reviewed and approved the study protocol (IRB approval number 0008-21-CMC). Written informed consent was obtained from the participants' parent included in the study. This article does not contain any studies with animals performed by any of the authors.

## Conflict of Interest Statement

Author G.L. declares grants and non-financial support from Vertex Pharmaceuticals, grants from Enanta Pharmaceuticals, grants from MedImmune, grants from DosentRx, outside the submitted work. Author K.Y. declares grants from Vertex Pharmaceuticals, grants from Gilead, outside the submitted work; Authors E.F., A.Z., N.P., I.O. declare that they have no conflict of interest.

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## Author Contributions

K.Y: supervision, conception, writing-drafting the initial manuscript. E.F: conceptualization/design and analysis and interpretation of the EBC results. A.Z: analysis of laboratory data. N.P. and I.O: acquisition of data. G.L: conceptualization/design, supervision, writing-review and editing of the manuscript. All authors read and approved the final submitted manuscript. All authors have agreed to be personally accountable for the author's own and others contributions.

## Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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