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Lung function assessment in children with Long-Covid syndrome

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Abstract

Introduction: A significant percentage of patients who survived the Coronavirus Infection Disease 2019 (COVID-19) showed persistent general and respiratory symptoms even months after recovery. This condition, called Post-Acute Sequelae of COVID-19 or Long-Covid syndrome (LCS), has been described also in children with positive history for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Little is known about the patho-physiologic mechanisms underlying this syndrome. The aim of this study was to investigate any difference between children with LCS and asymptomatic peers with previous COVID-19 in terms of lung function and lung ultrasound (LUS) patterns. Secondly, we tested associations between lung function abnormalities and LUS findings with Long-Covid.

Methods: We carried out a prospective, descriptive, observational study including 58 children aged 5–17 years: 28 with LCS compared to 30 asymptomatic children with previous COVID-19. We collected demographic data, history of asthma, allergy or smoke exposure, and acute COVID-19 symptoms. After a median period of 4.5 months (1%–95% range 2–21) since the infection, lung function was assessed by spirometry, body plethysmography, diffusion lung capacity for carbon monoxide (DLCO). Airways inflammation was investigated by fractional exhaled nitric oxide (FeNO). LUS was performed independently by two experienced clinicians.

Results: We found that children with LCS were older than controls (mean (SD) 12 (4.1) vs. 9.7 (2.6); $p = .04$). Children with LCS complained more frequently fatigue (46.4%), cough (17.9%), exercise intolerance (14.3%) and dyspnea (14.3%). Lung function was normal and similar between the two groups. The frequency of LUS abnormalities was similar between the two groups (43.3% children with LCS vs. 56.7% controls; $p = .436$). Children with LCS showed lower FeNO values (log difference −0.30 (CI 95% −0.50, −0.10)), but no association of LCS with a lower lung function and abnormal LUS findings was found.

Conclusions: LCS seems to be more frequent in older age children. Lung functional and structural abnormalities were not different between children with LCS and asymptomatic subjects with previous COVID-19. In addition, children with LCS showed lower FeNO values than controls, suggesting its potential role as a marker in LCS. However, further and larger studies are needed to confirm our findings.

KEYWORDS

lung function, lung ultrasound, pediatric Long-Covid, post-acute sequelae of COVID-19

1 | INTRODUCTION

During the last 3 years the entire world has faced the pandemic caused by Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), a novel form of Betacoronavirus.[1] To date, 646.740.524 confirmed cases and 6.637.512 deaths were reported to the World Health Organization.[2] Approximately 8.5% of the total cases are represented by children under 18 years old,[3] but this percentage has increased rapidly since the beginning of the pandemic, reaching 18.2% of cumulative cases in the United States of America.[4] After recovery from acute disease, several patients complain persistent symptoms involving different organs and fluctuating over time. This manifestation related to previous SARS-CoV-2 infection with no other medical explanation has been called Long-Covid syndrome (LCS) or Post-Acute Sequelae of COVID-19 syndrome.[5] Recent scientific reports show that LCS can also affect the pediatric population.[6–8] Although the clinical characteristics and the course of this condition seem to be similar in adults and children, little is known about the underlying pathophysiologic mechanisms. Adult LCS is characterized by the presence of pulmonary function tests (PFTs) and/or chest imaging abnormalities in about 30% of cases.[9] On the contrary, only two studies assessed PFTs in children with LCS and no relevant findings were reported.[10,11] A Czech multicenter study suggested that the long-term respiratory impact of COVID-19 was relatively mild with a favorable prognosis.[10] Dobkin et al.[11] showed that spirometry and plethysmography were normal in most children with LCS. The findings are contrasting due to the heterogeneity of the study population, different confounders and time evaluation of the lung function in follow-up visit. Pulmonary sequelae of COVID-19 in children have been studied also using lung ultrasound (LUS), which shows the advantage of being easily available, fast, simple, radiation-free, and relatively cheap.[12] La Regina et al.[13] screened using LUS 607 children with previous SARS-CoV-2 infection and found LUS abnormal findings only in a small subgroup of children. However, the authors did not collect data on Long-Covid symptoms in this study population.

Hence, we carried out this prospective descriptive observational study which compared children with Long-Covid symptoms and asymptomatic ones with a history of SARS-CoV-2 infection. The study aim was to investigate the associations of LCS with PFTs and LUS findings.

2 | MATERIALS AND METHODS

2.1 | Subjects and study design

In this prospective descriptive observational study, we recruited from May 2021 to October 2022 children aged 6–18 years with SARS-CoV-2 infection in the previous 24 months who attended the Pediatric Allergy and Pulmonology Unit of the Department of Pediatrics, University of Chieti, Italy. Additionally, the study population was divided into children with and without LCS, considering children without Long-Covid symptoms as our control group. LCS was defined according to Delphi-consensus definition which included the presence of symptoms lasting more than 12 weeks from the acute infection.[14] We excluded patients with chest or skeletal deformities, cystic fibrosis, neuromuscular disorders, immunodeficiency, cardiac and autoimmune diseases, malignancies, chronic infectious diseases, and congenital disorders.

We created a list of suitable subjects from the schedules archive and invited their parents by phone to participate in the study.

All children sequentially underwent spirometry, body plethysmography, diffusing capacity of lung for carbon monoxide (DLCO) and fraction of exhaled nitric oxide (FeNO). LUS was performed after PFTs and before the bronchodilator test (BDT).

Written informed consent was obtained from parents or legal representatives of the participants; children >12 years of age were asked to give also their assent. The study was approved by the local Ethical Committee of the University of Chieti, and it was conducted in compliance with ethical principles based on the Declaration of Helsinki.

2.2 | Data collection

A research assistant collected data on maternal and child characteristics of the study population by questionnaires. Anthropometric measurements (height, weight, body mass index [BMI]) were measured at the center during the visit and were expressed as standard deviation score (SDS) using the Italian Society of Pediatric Endocrinology and Diabetology growth calculator program according to Cacciari et al[15] Additionally, we collected information on the previous acute COVID-19 and LCS. Regarding LCS, we asked about

the persistence of the following symptoms: fatigue, headache, dyspnea, cough, exercise intolerance, cross-eyed, wheezing, rhinitis, paranesthesia, myalgia, arthralgia, dizziness, attention deficits, loss of memory, ageusia, anosmia, palpitation, chest pain, diarrhea, vomit, and recurrent abdominal pain.

2.3 | Pulmonary function tests, airway inflammation investigation and lung ultrasound protocol

According to the American Thoracic Society(ATS)/European Respiratory Society (ERS),[16] lung function was assessed by spirometry (VyntusTM, Jaeger® IM PRO, Carefusion, Germany 234 GmbH). In an upright position with a nose-block clip applied, the patients underwent a spirometric examination for three consecutive technically acceptable maneuvers. The spirometric parameters were forced expiratory volume at 1 s (FEV_1), forced vital capacity (FVC), and FEV_1/FVC ratio and were converted into gender-, height-, and age-adjusted z-scores according to the Global Lung Initiative reference data.[17] The lung function evaluation was performed by a pediatric pulmonologist and the best spirometric measurements were considered for statistical calculations. Acceptable repeatability was achieved when the difference between the largest and the next largest FVC was 0.150 L and the difference between the largest and next largest FEV1 was 0.150 L, as reported by Graham et al.[18]

Specific airway resistance (SRaw), total lung capacity (TLC) and residual volume (RV) were measured using standardized body plethysmography (Vmax Autobox V62J; Sensor Medics, CareFusion) according to Hall et al.[19] The patient was instructed to attach to the mouthpiece and breathe quietly until a stable end-expiratory level was achieved. When the patient was at or near functional residual capacity (FRC), the shutter was closed at end-expiration for 2–3 s, and the patient was instructed to perform a series of gentle pants at a frequency between 0.5 and 1.0 Hz. A series of 3–5 technically satisfactory panting maneuvers were recorded, after which the shutter was opened and the patient performed an expiratory reserve volume maneuver, followed by a slow inspiratory vital capacity maneuver. At least three FRC values that agreed within 5% (i.e., difference between the highest and lowest value divided by the mean is ≤0.05) were obtained and the mean value reported.

DLCO was measured with a standardized single breath technique (Vmax® Autobox V62J, Carefusion, Hoechberg, Germany). Hemoglobin concentration was assumed to be normal as no child in the study had a medical history of anemia. After establishing a stable breathing pattern, the patient was instructed to exhale completely. The valve to the test gas was opened and the child took maximum inspiration. The maneuver was considered reliable if the inspiratory time was <4 s and the inhaled volume was at least 85% of the largest vital capacity. The patient then held the breath for 10 ± 2 s, maintaining near atmospheric pressure during the apnea (performing neither a Valsalva nor Muller maneuver). DLCO maneuvers were repeated until at least two technically acceptable and reproducible tests were obtained.[20]

Additionally, DLCO and lung volumes parameters were converted into gender-, height-, and age- adjusted z-scores according to the Global Lung Initiative reference data.[19,21]

FeNO was assessed with an on-line method using a single breath exhalation and a sensitive chemiluminescence assay (Ecomedics CLD 88), according to ATS/ERS recommendations.[22] The mean of three readings at the end of the expiration (plateau phase) was taken as the representative value for each measurement.

BDT was carried out 15 min after the administration of salbutamol (400 mcg) using a spacer. An increase of at least 12% in FEV_1 was considered significant for bronchial reversibility.[23]

LUS was separately performed by two pediatric sonographers, each blinded to the other's findings, The two sonographers attended dedicated LUS courses and shared similar experience in this field. Accessible lung fields were visualized using commercial ultrasound sonography (Samsung HM70A, Republic of Korea, 2013) equipped with a 3–16 MHz linear probe. As described by Copetti and Cattarossi,[24] the probe was placed perpendicular and parallel to the ribs to view the intercostal spaces. The sonographer slowly examined the chests of the patients while seated, upright, or in the parent's holding position. Each hemithorax was divided into six segments: anterior (parasternal to anterior axillary line), lateral (anterior to posterior axillary line), and posterior (posterior axillary line to paravertebral), with each of these three subdivided into superior and inferior segments. Every visualized section of the lung was assessed for normal or abnormal echogenic appearance. If a zone was considered abnormal, the sonographer recorded the specific findings encountered: the absence of lung sliding (the absence of respiration movement between the visceral and parietal pleura); ≥3 B-lines-laser-like vertical hyperechoic reverberation artifacts that arise from the pleural line to the bottom of the image; pleural effusion-anechoic space between the visceral and parietal pleura to the bases of the lungs; micro-consolidation and macro-consolidation-pleural-echo-poor or tissue-like region, respectively less and greater than 1 cm; pleural line irregularity-irregular, thickened, or fragmented pleural line. The LUS was defined as positive in the presence of one or more of the aforementioned findings in any of the subject's lung zones.

2.4 | Statistical analysis

Continuous data was expressed as mean (standard deviation [SD]) or median (5%–95% range), and categorical data was presented as percentage and count. We used logarithmic transformation for normalizing the skewed distribution of the continuous data. We compared characteristics of the study groups by using unpaired T-tests, Mann-Whitney U tests, and Pearson's Chi-square tests. We evaluated if LCS was associated with lung function parameters and LUS findings by using a multivariable linear and logistic regression analysis. Confounders were selected from literature first, and were subsequently tested for their association with both the determinant and the outcome, or a change of the unadjusted effect estimates of 10% when added to the univariate model.[25] The potential

confounders which we selected from the literature were gender, age, asthma comorbidity, allergic diseases, smoke exposure, BMI, and physical activity.[26–30] Hereafter, confounders were included in the final model if they were either associated with exposure (LCS) and outcome (respiratory investigation), and not in the causal pathway, or if the effect estimate changed by 10% when they were included. We created a directed acyclic graph (DAG) to better visualize the relationship between exposure, outcome and potential confounders (Figure S1). All measures of association were presented as z scores or absolute measured values difference, or odds ratios and their corresponding 95% confidence intervals. Statistical analyses were performed using SPSS version 25.0 for Windows software (IBM Corp) and STATA/IC 15.1 (StataCorp LLC 4905 Lakeway Drive College Station, Texas 77845-4512, USA).

3 | RESULTS

Of the 70 eligible children, 64 participated in the study. Four children met the exclusion criteria and two were lost to follow-up visit. Fifty-eight subjects were included in the statistical analysis. Specifically, 28 complained Long-Covid symptoms and 30 children were asymptomatic (control group). Flow-chart of the study is shown in the Figure 1.

General characteristics of the study population are shown in the Table 1.

The two groups were comparable for gender and pubertal development. Children with LCS were older than the control group (12.0 (4.1) vs. 9.7 (2.6), $p = .04$), although no difference was found for height, weight and BMI between the two groups. In the Long-Covid group, 11 (39.3%) children showed current asthma and 20 (71.4%) were atopic. No difference was found for asthma or allergy prevalence between the two groups, as well as in asthma severity and control.

Additionally, among children with asthma ($n = 30$ (51.7%), we found no difference between before and after COVID-19 for FEV_1 z score (mean (SD), 0.8 (1.0) vs. 0.8 (1.8), respectively, $p = .959$) and FVC z score (1.0 (1.0) vs. 1.3 (1.8), respectively, $p = .551$), while a difference was observed for FEV_1/FVC z score, although weakly significant (1.5 (2.7) vs. 0.2 (1.7), respectively, $p = .047$) (Table S1). Using a simple logistic regression analysis, we observed that the presence of asthma was not associated with an increased risk to develop Long-Covid symptoms (ORs (CI 95%), 1.15 (0.41, 3.24)). In addition, that association remained not significant even after adjustment for potential confounders (1.09 (0.36, 3.36)). We also found no difference for spirometric parameters, TLC, airway inflammation and lung diffusion capacity between children with cough and/or dyspnea as Long-Covid symptoms. The only difference was found for RV between children with or without cough and/or dyspnea, although it was not clinically relevant (not above upper limit of normal)[31] (Table S2).

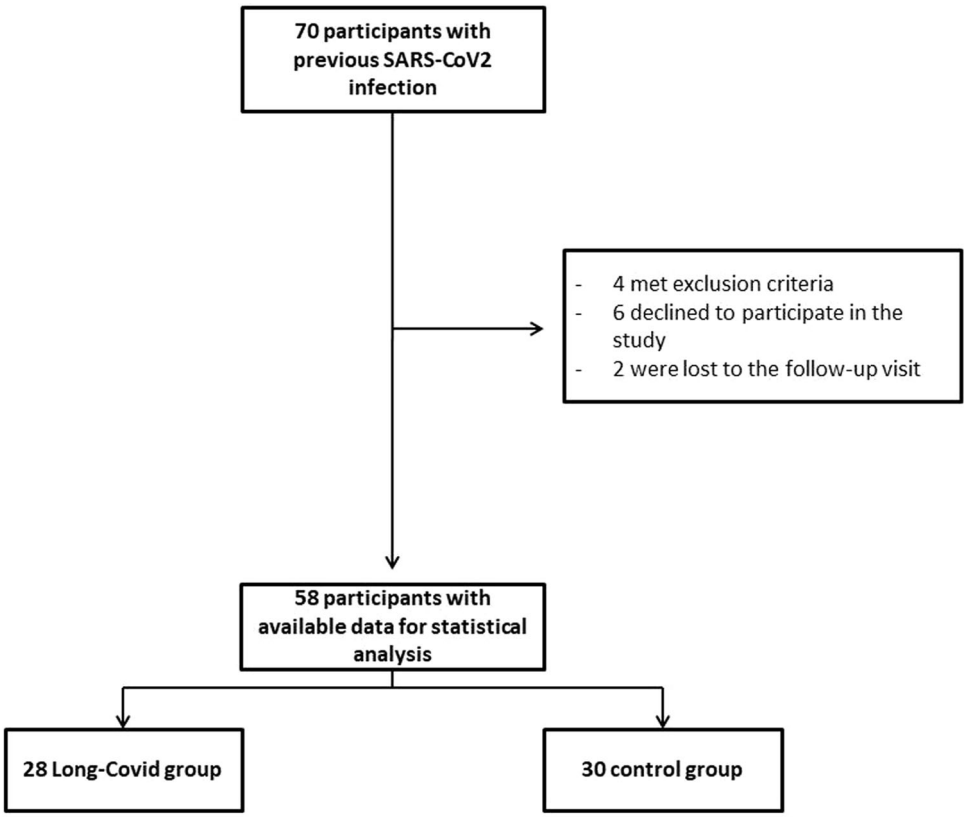

FIGURE 1 Flow-chart of the study.

TABLE 1 General characteristics of the general population

	Long-Covid group $n = 28$	Control group $n = 30$	p value
Gender, female (%)	12 (42.9)	16 (53.3)	.425
Age at assessment (year)	12.0 (4.1)	9.7 (2.6)	.040*
Height (SDS)	0.1 (0.7)	−0.4 (1.2)	.050*
Weight (SDS)	0.6 (1.1)	0.1 (1.2)	.835*
BMI (SDS)	0.8 (1.2)	0.4 (1.0)	.322*
Pubertal onset, yes (%)	16 (55.2)	13 (44.8)	.293
Ever asthma, yes (%)	15 (53.6)	15 (50.0)	.786
Current asthma, yes (%)[a]	11 (39.3)	9 (30.0)	.643
Allergic rhinitis, yes (%)	16 (57.1)	20 (66.7)	.455
Atopic dermatitis, yes (%)	11 (39.3)	13 (43.3)	.754
Smoke exposure, yes (%)	13 (46.4)	14 (46.7)	.986
Skin prick test, yes (%)	20 (71.4)	22 (73.3)	.704
Antiasthma therapy, (%)	19 (67.9)	18 (60.0)	.534
– *ICS*	12 (42.9)	11 (36.7)	.786
– *Antileukotriene*	1 (3.6)	0 (0.0)	1.000
– *OCS*	0 (0.0)	3 (10.0)	.236
Antiallergy therapy, (%)			
– *Nasal corticosteroids*	11 (39.3%)	9 (30.0%)	.577
– *Antihistamine*	18 (64.3%)	16 (53.3)	.584
Severity asthma, (%)			
– *Mild*	8 (72.7)	6 (66.7)	1.000
– *Moderate*	3 (27.3)	3 (33.3)	1.000
– *Severe*	0 (0.0)	0 (0.0)	-
Patients with symptomatic COVID-19, yes (%)	27 (96.4)	23 (76.7)	.053
Symptoms in COVID-19, yes (%)			
– *Fever*	13 (46.4)	15 (50.0)	.786
– *Rhinitis*	9 (32.1)	4 (13.3)	.086
– *Cough*	12 (42.9)	10 (33.3)	.455
– *Dyspnea*	5 (17.9)	2 (6.7)	.246
– *Anosmia*	4 (14.3)	1 (3.3)	.187
– *Ageusia*	3 (10.7)	2 (6.7)	.665
– *Sore throat*	2 (7.1)	3 (10.0)	1.000
– *Nausea*	1 (3.6)	1 (3.3)	1.000
– *Diarrhea*	4 (14.3)	0 (0.0)	.048
– *Arthralgia*	1 (3.6)	1 (3.3)	1.000
– *Myalgia*	4 (14.3)	3 (10.0)	.701
– *Anorexia*	6 (21.4)	3 (10.0)	.290
– *Headache*	5 (17.9)	7 (23.3)	.607
Number of symptoms ≥ 3 in COVID-19, yes (%)	12 (42.9)	9 (30.0)	.309
Symptoms in Long-Covid, yes (%)			
– *Fatigue*	13 (46.4)		
– *Headache*	3 (10.7)		
– *Dyspnea*	4 (14.3)		
– *Cough*	5 (17.9)		
– *Exercise intolerance*	4 (14.3)		
– *Wheezing*	3 (10.7)		
– *Rhinitis*	2 (7.1)		
– *Cross-eyed*	1 (3.6)		
– *Neurological problems*	1 (3.6)		
– *Arthralgia*	1 (3.7)		
– *Myalgia*	2 (7.1)		
– *Cognitive symptoms*	2 (7.1)		

Note: Data are expressed as means (SD), medians and range, percentages and absolute numbers; SDS=standard deviation score; BMI=Body Mass Index; ICS=Inhaled Corticosteroids; OCS=Oral Corticosteroids.

[a]current asthma was defined as ever diagnosis of physician diagnosed-asthma, obtained by parental or self-reported questionnaires at the visit, with either wheezing or medication use in the past 12 months. Bold p value < .05; p value obtained from Chi squared test.

*p value from Unpaired t test.

Furthermore, we observed that children abnormal PFTs showed no difference in terms of percentage of LCS, age, gender, BMI SDS, atopic sensitization, and asthma prevalence than children with normal PFTs (Table S3).

The most frequent symptoms during acute COVID-19 were fever (46.4% in the Long-Covid group vs. 50.0% in the control group, $p = .786$), cough (42.9% vs. 33.3%, respectively, $p = .455$) and rhinitis (32.1% vs. 13.3% respectively, $p = .086$). Almost all children with LCS had at least one symptom during acute infection and more than 20% of the children in the control group was asymptomatic. LCS in our population was characterized mainly by fatigue (46.4%), cough (17.9%), exercise intolerance (14.3%), dyspnea (14.3%), headache (10.7%), and wheezing (10.7%) (Table 1).

From univariate analysis, we found no difference for lung function parameters, diffusion lung capacity or airways inflammation between the two groups (Table 2). LUS showed abnormal findings in 43.3% of the Long-Covid group and 56.7% of the control group. The most frequent findings were multiple B-lines (36.2%), whereas no pleural effusion or lung consolidation larger than 1 cm were detected in both groups. However, no difference for LUS findings was found between the two groups (Table 2).

TABLE 2 Lung function and ultrasound characteristics of the study population

	Long-Covid group *n* = 28	Control group *n* = 30	*p* value
FEV_1 z score	0.3 (1.4)	0.8 (1.5)	.201*
FVC z score	0.5 (1.5)	0.9 (1.4)	.341*
FEV_1/FVC z score	1.0 (2.6)	0.7 (1.5)	.591*
sRaw (%)	130 (46–288)	147 (52–394)	.213[a]
missing	*n* = 4	*n* = 3	
RV z score	−0.24 (−0.1)	−0.41 (0.9)	.492*
missing	*n* = 6	*n* = 9	
TLC z score	0.2 (1.1)	0.1 (1.1)	.625*
missing	*n* = 6	*n* = 9	
DLCO_SB z score	0.7 (1.2)	0.8 (1.0)	.835*
missing	*n* = 11	*n* = 6	
FeNO (ppb)	7.3 (2.1–23.0)	11.0 (4.2–36.2)	.052[a]
missing	*n* = 5	*n* = 11	
LUS findings, yes (%)	13 (43.3)	17 (56.7)	.436
LUS patterns			
– *B lines*	8 (28.6)	13 (41.3)	.242
– *Consolidation < 1 cm*	2 (7.1)	8 (26.7)	.081
– *Consolidation > 1 cm*	0 (0.0)	0 (0.0)	-
– *Pleural abnormalities*	8 (28.6)	10 (33.3)	.695
– *Pleural effusion*	0 (0.0)	0 (0.0)	-

Note: Data are expressed as means(SD), medians and range, percentages and absolute numbers; FEV1= Forced Expiratory Volume in 1 s; FVC=Forced Vital Capacity; DLCO_SB= Single-Breath Diffusing Capacity of the Lung for Carbon Monoxide; sRaw = specific airway resistance; RV=Reserve Volume; TLC=Total Lung Capacity; FeNO=Fraction of Exhaled Nitric Oxide; ppb=parts per billion; LUS=Lung Ultrasound. Bold *p* value < .05; *p* value obtained from Chi squared test.

[a]*p* value obtained from Mann-Whitney U test;

*p value from Unpaired t test

Confounder model showed no association of LCS with spirometric parameters: FEV_1 z score (z score difference [Confidence Interval 95%], −0.46 (−1.30, 0.38)), FVC z score (−0.45 (−1.30, 0.39)), and FEV_1/FVC z score (0.05 (−1.16, 1.26)). No association was also found between LCS and specific airway resistance or lung volumes: log absolute number difference (CI 95%), −0.04 (−0.17, 0.09)), RV z score (z score difference (CI 95%), 0.52 (−0.03, 1.07)) and TLC z score (0.50 (−0.29, 1.28)) (Table 3). Similarly, we found no association between LCS and DLCO z score (0.15 (−0.67, 0.96). Noteworthy, we found that log FeNO values were lower in the Long-Covid group than the control group (−0.30 (−0.50, −0.10)) (Table 4). Lastly, we found no associations of LCS with LUS findings (0.88 (0.27, 2.86)) (Table 4).

4 | DISCUSSION

This study compared a group of children complaining Long-Covid symptoms and a group of children who completely recovered after an acute SARS-CoV-2 infection, aiming to find a respiratory pathophysiological pattern underlying this condition. We showed lower log FeNO values in children with Long-Covid symptoms than controls, although no associations of LCS with lung function parameters and ultrasound findings were found. In our study children with LCS were older than controls. This finding is consistent with a recent meta-analysis including 80,071 children and adolescents with LCS, which showed a higher prevalence of Long-Covid symptoms in older age groups.[32]

Female gender, BMI, and asthma and allergy prevalence in our study population were similar between the two groups. Contrarily, in a meta-analysis, Lopez-Leon et al[32] found that female, overweight/obesity, allergic diseases and more severe COVID-19 were additional risk factors for developing LCS. More probably, those contrasting findings could be due to the difference in sample size and the heterogeneity of the study population. Additionally, we did not investigate the effect of COVID-19 severity on LCS risk given that the most of our subjects showed a mild COVID-19.

The most frequent Long-Covid symptoms in our study population were fatigue (46.4%), cough (17.9%), exercise intolerance (14.3%), dyspnea (14.3%), headache (10.7%) and wheezing (10.7%). Rhinitis and cognitive symptoms were reported in 7.1% of the subject. According to our findings, Lopez-Leon et al[19] showed as frequent clinical manifestations of LCS, fatigue (9.7%), headache (7.8%), respiratory symptoms (7.6%), nasal congestion (7.5%) and rhinorrhea (4.2%), and cognitive symptoms (6.3%). Contrarily, the prevalence of the neurological problems was higher in our study population compared to the aforementioned meta-analysis (3.6% vs. 0.9% respectively). However, the prevalence of Long-Covid symptoms strongly depends on the follow-up time since acute COVID-19. The follow-up time since the acute infection in the meta-analysis ranged from 1 to 13 months,[19] while in our study it ranged from 2 to 21 months. Dolezalova et al[10] found that the median recovery time from an acute infection was 4 months (range 1.5–8.0). We hypothesized that recall bias could have affected those findings.

Additionally, among children with asthma, we found no difference between before and after COVID-19 for FEV_1 z score and FVC z score, while a difference was observed for FEV_1/FVC z score, although weakly significant. However, no association was found between the presence of asthma and the increased risk of developing Long-Covid symptoms. This is different from what was reported in adults regarding the risk factors for long COVID which included female sex, middle age, and comorbidities, especially asthma.[33] Unfortunately, further studies are needed to confirm the association with asthma, mostly in children and adolescents. We found no association of LCS with lung function parameters and diffusion capacity. Our findings are consistent with those of several studies in the literature.[10,11] In particular, a Czech multicenter study including 39 children with a median age of 13 years with LCS found no

TABLE 3 Associations of Long-Covid Syndrome with lung function parameters

	FEV_1 z score Difference (CI 95%) *n* = 58	FVC z score Difference (CI 95%) *n* = 58	FEV_1/FVC z score Difference (CI 95%) *n* = 58	TLC z score Difference (CI 95%) *n* = 43	Log sRaw (kPas)[a] Difference (CI 95%) *n* = 51	RV z score Difference (CI 95%) *n* = 43
(Long-Covid vs. Control)						
Crude model*	−0.25 (−1.17, 0.66)	−0.11 (−1.04, 0.82)	0.30 (−0.81, 0.41)	1.16 (−0.51, 0.83)	−0.02 (−0.13, 0.09)	0.18 (−0.34, 0.69)
p value	.570	.810	.591	.625	.719	.492
Confounder model**	−0.46 (−1.30, 0.38)	−0.45 (−1.30, 0.39)	0.05 (−1.16, 1.26)	0.50 (−0.29, 1.28)	−0.04 (−0.17, 0.09)	0.52 (−0.03, 1.07)
p value	.273	.288	.936	.207	.523	.060

Note: Data are presented as change in Z-score difference or logarithmic absolute values derived from linear regression model with their corresponding 95% confidence intervals.

Abbreviations: FEV_1= forced expiratory volume in 1 s; FVC= forced vital capacity; sRaw= Specific Airway Resistance; RV=Residual Volume; TLC=Total Lung Capacity. [a]Crude model was obtained considering the effect of only one independent variable (Long-Covid diagnosis).

*We applied logarithmic transformation for normalizing skewed distribution of sRaw absolute number.

**Confounder model adjusted for sex, age, and BMI. Bold: *p* value < .05.

TABLE 4 Associations of Long-Covid syndrome with airway inflammation, lung diffusion capacity and ultrasound abnormalities

	Log FeNO (ppb) Difference (CI 95%) *n* = 42	DLCO z score Difference (CI 95%) *n* = 41	LUS OR (CI 95%) *n* = 58
(Long-Covid vs Control)			
Crude model*	−0.20 (−0.38, -0.02)	−0.07 (0.76, 0.62)	0.66 (0.24, 1.87)
p value	**.035**	**.835**	.436
Confounder model**	−0.30 (−0.50, -0.10)	0.15 (−0.67, 0.96)	0.88 (0.27, 2.86)
p value	**.004**	.713	.834

Note: Data are presented as change in logarithmic absolute values or z score difference, and odds ratio derived from linear and logistic regression models, respectively, with their corresponding 95% confidence intervals; [a]We applied logarithmic transformation for normalizing skewed distribution of FeNO absolute number.

Abbreviations: FeNO=fractional exhaled nitric oxide; DLCO= diffusing capacity of the lung for carbon monoxide.

*Crude model was obtained considering the effect of only one independent variable(Long-Covid diagnosis).

**Confounder Model was adjusted for sex, age, and BMI. Bold: *p* value < .05.

association of Long-Covid symptoms with a lower lung function and abnormal chest X-ray findings, although a mild decline in DLCO was found in about 11% of the children.[10] Dobkin et al.[11] found normal spirometry, plethysmography and DLCO values in a cohort of 29 children aged 13 years with LCS evaluated about 3 months after acute infection. These findings suggested that lung inflammation during acute COVID-19 in children was generally mild and unable to induce fibrotic change which could persistently affect the lungs.

In adults, 36% of the subjects with LCS showed subjective dyspnea and exercise intolerance measured by 6-min walking test, although the lung function (FEV_1, FVC, TLC, or DLCO) remained normal.[34] Similarly, Rinaldo et al.[35] studied a cohort of 91 adult COVID-19 survivors by cardiopulmonary exercise testing, with the most frequently observed abnormality consisting in early anaerobic threshold likely induced by muscle deconditioning. In the absence of permanent functional abnormalities, symptoms such as fatigue and exercise intolerance might be explained by muscle deconditioning, and behavioral changes induced by lockdown and social isolation.[36] In a prospective cohort study, including 518 Russian children aged 10 years with previous SARS-CoV-2 infection, 5% of the parents reported emotional and behavioral changes of their children, such as changes in eating habits, emotional wellbeing and physical activities.[37] Indeed, 10% of the children were spending more time on social-media and virtual platforms for educational and recreational purposes, avoiding face-to-face communication with their peers.[37]

To the best of our knowledge, this is the first study measuring FeNO levels in children with LCS. In confounder model, we found lower FeNO values in children with LCS than those without Long-Covid symptoms. Nitric oxide levels in airways is derived from epithelial inducible nitric oxide synthase (iNOS), whose activity is triggered by pro-inflammatory cytokines.[38] FeNO levels reflect nitric oxide derived from the proximal airways, while alveolar concentration

of exhaled nitric oxide (CaNO) represents the nitric oxide produced in the distal airways including the bronchioloalveolar spaces.[39] CaNo measurement requires the multiple-flow technique FeNO test which is more difficult to be performed. Instead, in our study we used the single breath measurement because more suitable for the children, and thus we could only assess nitric oxide levels in proximal airways. To date, the influence of SARS-CoV-2 infection on FeNO levels is controversial and investigated only in adults. In 82 adult patients hospitalized for COVID-19, Betancor et al.[38] found normal range values of FeNO, independently from disease severity or patient's history of atopy. Interestingly, FeNO values were lower during acute infection while increased during the recovery phase. The authors attributed this fluctuation to the administration of corticosteroids during acute infection, which inhibit nitric oxide production by iNOS. Adult patients with a history of severe COVID-19 showed higher CaNO values but normal FeNO levels a few months after hospital discharge.[40,41] Specifically, Cameli et al.[40] observed higher values of CaNO about 90 days after the hospital discharge in 20 post-COVID-19 patients than healthy gender- and-age matched controls, although FeNO levels were normal in both. Similarly, Hua-Huy et al.[41] found no different FeNO levels among patients with mild, moderate and severe COVID-19-related lung disease 5 months after acute infection. However,, the authors observed increased CaNO levels in the severe/critical disease group compared to the mild disease one. Contrarily, Lindahl et al.[42] found normal FeNO and CaNO levels in adult patients previously hospitalized for severe COVID-19 at 3-6 months from the recovery.[36] Nitric oxide has an antimicrobial action versus bacteria, fungi, parasites and selected viral species, and its production increases during viral infections.[43] Hence, we speculated that lower levels of FeNO might have favored the viral persistence in the respiratory airways even months after the acute infection, inducing low levels of the immune activation.[44] However, the role of nitric oxide synthesis and concentration during viral infections is still a matter of debate and further larger studies are needed to clarify the mechanism underlying that association.

The usefulness of LUS in detecting lower respiratory tract involvement in children with SARS-CoV-2 acute infection is well known.[45] In an observational study including eight children during acute COVID-19, Denina et al.[46] firstly demonstrated a concordance of LUS findings with radiologic findings in seven out of eight patients, suggesting that LUS may be a reliable method to detect lung abnormalities in children with COVID-19. However, the role of LUS in follow-up assessment is still unclear. We chose LUS as the main imaging tool to investigate structural abnormalities over time as it is a noninvasive nonionizing radiation and well-tolerated tool mostly in pediatric population.[47] In our study, we observed no difference for LUS findings between the two groups.

An Italian observational study conducted on a cohort of 607 children with a previous SARS-CoV-2 infection described LUS abnormal findings in a few patients, mainly pleural line irregularities (27%), B-lines (17%), and small subpleural consolidations (9%).[13] Noteworthy, the frequency of artifacts decreased with increasing time since the acute infection. Patients symptomatic during the acute infection presented multiple B-lines more frequently than asymptomatic ones (19% vs. 10%).

According to our findings, in a prospective study including 647 children aged 8 years, Buonsenso et al.[45] observed persistent symptoms in about 40% of patients 2 months after acute infection. Importantly, LUS was mostly normal in all children showing similar findings between children with and without LCS.

Recently, Grager et al.[48] found no significant difference in the morphological LUS findings in 30 children with LCS than 15 healthy children.

We hypothesized that LUS findings might normalize over time with a complete disappearance of sonographic alterations and a total lung recovery during the follow-up. Additionally, we suggested that LUS might not be used as a reliable tool to discriminate children with LCS from those asymptomatic. Likely, the cause of the persistent respiratory symptoms in LCS should not be related to lung morphological alterations detectable with this technique.

There is increasing evidence that Long-Covid features are similar to other post-viral chronic fatigue syndromes, whose pathogenesis is not yet clear. The underlying LCS pathogenesis might involve functional mechanisms rather than organ damage.[49] Indeed, in our study population the lack of difference in lung function and structure between the two groups supports this hypothesis.

Chronic stimulation of the immune responses, viral persistence, gut dysbiosis, endothelial inflammation of peripheral microcirculation and microclots, and mitochondrial dysfunction were hypothesized as underlying mechanisms of Long-Covid.[49] We speculated that the viral persistence mechanism might be facilitated from lower FeNO values given its antimicrobial action, although we cannot establish the causal pathway for our study design.

The main strength of the study is that it is the first pediatric study comparing FeNO levels, PFTs, and LUS between children with LCS and asymptomatic children with previous SARS-CoV-2 infection. This allowed us to investigate any associations of Long-Covid symptoms with both lung functional and morphological findings. Second, we used appropriate statistical methods evaluating the effect of potential confounding factors. Third, lung function measurements were performed by the same operator with expertize in the field of lung diseases. Lastly, two experienced operators performed independently LUS scans to minimize bias related to the ultrasonographer experience. However, several methodological limitations need to be discussed. First, the small sample size could have affected the power of the study, although comparable to other studies in the literature.[10,11] Second, we decided to perform basal spirometry, plethysmography and DLCO tests before FeNO as they required a higher degree of compliance, although forced spirometry maneuvers could have affected the NO concentration in expired air.[22] However, the PFTs order of execution was the same for both groups, therefore the difference in FeNO findings was still of interest. Third, the participants were recruited at the Pediatric Allergy and Pulmonology Unit determining the high prevalence of asthmatic and atopic children in our study population. Thus, the findings might not be generalized to a general population. However,

the two groups were homogeneous in terms of prevalence of current asthma and atopy, asthma severity and treatments. Additionally, we had no lung function and structure assessment during the acute infection. Therefore, we might have missed any functional or morphological alterations during the acute infection and their changes over time. A larger sample and multiple follow-up visits would point out the change of the symptoms over time, including the time interval from the acute infection to the onset of the Long-Covid symptoms and the disappearance of themselves.

5 | CONCLUSIONS

Lung functional and structural findings were similar between children with Long-Covid symptoms and those with a complete recovery after SARS-CoV-2 infection. Additionally, we found lower FeNO values in children with Long-Covid symptoms than controls, suggesting a possible implication of airways NO production in Long-Covid pathogenesis However, further larger studies are needed to better investigate the association of LCS with bronchial inflammation and characterize lung functional and structural alterations in LCS.

AUTHOR CONTRIBUTIONS

Marina Attanasi and Francesco Sansone conceived the idea. Francesco Sansone, Daniele Russo, Paola Di Filippo, and Laura Sgrazzutti worked on the data collection. Marina Attanasi and Francesco Sansone reviewed the database. Marina Attanasi analyzed the database. Francesco Sansone, Marina Attanasi, and Paola Di Filippo wrote the first draft of the paper. All authors contributed to data interpretation and reviewed the manuscript. Marina Attanasi, Francesco Chiarelli, and Sabrina Di Pillo critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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