Characteristics and predictors of Long Covid in children: a 3-year prospective cohort study

Anna Camporesi,[a] Rosa Morello,[b] Anna La Rocca,[b,c] Giuseppe Zampino,[b] Federico Vezzulli,[d] Daniel Munblit,[e,f] Francesca Raffaelli,[g] Piero Valentini,[b] and Danilo Buonsenso[b,h,i,*]

[a]Pediatric Anesthesia and Intensive Care, V. Buzzi Children's Hospital, Milano, Italy
[b]Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[c]Medicine ans Surgery, Università Cattolica del Sacro Cuore, Rome, Italy
[d]Department of Pathophysiology and Transplantation, University of Milano, Milano, Italy
[e]Care for Long Term Conditions Division, Florence Nightingale Faculty of Nursing, Midwifery and Palliative Care, King's College London, London, United Kingdom
[f]Department of Paediatrics and Paediatric Infectious Diseases, Institute of Child's Health, Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation
[g]UOC Malattie Infettive – Dipartimento Scienze Mediche e Chirurgiche -. Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[h]Centro di Salute Globale, Università Cattolica del Sacro Cuore, Rome, Italy
[i]Area Pediatrica, Dipartimento di Scienze della Vita e Sanità Pubblica, Università Cattolica del Sacro Cuore, Rome, Italy

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Summary

Background Children can develop Long Covid, however long term outcomes and their predictors are poorly described in these patients. The primary aim is to describe characteristics and predictors of Long Covid in children assessed in-clinics up to 36 months post-SARS-CoV-2 infection, as well as investigate the role of vaccines in preventing Long Covid, risk of reinfections and development of autoimmune diseases.

Methods Children aged 0–18 years old with confirmed SARS-CoV-2 infection were invited for a prospective follow-up assessment at a peadiatric post-covid clinic in Rome, Italy, at serial intervals (3-, 6-, 12-, 18-, 24- and 36-months post-infection onset, between 01/02/2020 and 28/02/2024). Long Covid was defined as persistence of otherwise unexplained symptoms for at least three months after initial infection.

Findings 1319 patients were initially included, 1296 reached the 3 months follow-up or more. Of the patients who underwent multiple follow-ups, 23.2% (301), 169 (13.2%), 89 (7.9%), 67 (6.1%), 47 (7.1%) were diagnosed with Long Covid at 3-6-12-18-24 months, respectively For the primary outcome of Long Covid at three months, age >12 years (P < 0.001, OR 11.33, 95% CI 4.2; 15.15), comorbidities (P = 0.008, OR 1.83, 95% CI 1.06; 2.44), being infected with original variants (P < 0.001, OR 4.77, 95% CI 2.46; 14.47), female sex (P < 0.001, OR 1.62, 95% CI 1.02; 1.89) were statistically significant risk factors. Age >12 years (P = 0.002, OR 9.37, 95% CI 1.58; 8.64), and infection with original (P = 0.012, OR 3.52, 95% CI 1.32; 8.64) and alfa (P < 0.001, OR 4.09, 95% CI 2.01; 8.3) SARS-CoV-2 variants remained statistically significant risk factors for Long Covid duration for at least 18 months. Vaccination was associated with a lower risk of long covid at 3, 6 and 12 months for older children and a lower risk of reinfections. Being infected with the original SARS-CoV-2 variant was associated with a higher risk of new-onset autoimmune diseases ((P = 0.035, 95% CI 1.12; 2.4). One patient was diagnosed with Long Covid after a re-infection.

Interpretation This is the longest follow-up study of children with SARS-CoV-2 infection, showing a significant and long-lasting burden of Long Covid in the pediatric population. Our findings highlight the urgent need of investing in pediatric Long Covid in order to find effective diagnostic and therapeutic approaches, as well can inform preventive strategies in case of future pandemics.

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*Corresponding author. Largo A. Gemelli 8, 00168, Rome, Italy.
E-mail addresses: danilobuonsenso@gmail.com, danilo.buonsenso@policlinicogemelli.it (D. Buonsenso).
@danilo.buonsenso_surf4children (D. Buonsenso).

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Research in context

Evidence before this study

The majority of pediatric studies evaluating the long term impact of COVID-19 in children has mostly focused on the administration of online surveys to parents or children following SARS-CoV-2. In addition, only two pediatric studies performed a systematic follow-up after initial infection, still using online surveys and only a few timepoints. None of published studies assessed patients in clinical settings using long-term standardised medical assessments (up to thirty six months following the initial infection) and exclusion of other alternative diagnoses, to inform the Long Covid diagnosis and definition. In additions, while some other studies defined the relationship with different SARS-CoV-2 strains and vaccination status on the risk of developing Long Covid, no other pediatric studies have evaluated the risk of developing new autoimmune conditions or the risk of developing long Covid after reinfections.

Added value of this study

To the best of our knowledge, this is the first study to have prospectively assessed a prospective cohort of children with microbiologically confirmed SARS-CoV-2 infection for up to 36months post-Sars-CoV-2 infection, as well the only evaluating the risk of developing new autoimmune conditions or the risk developing long Covid after reinfections. Children were assessed by physicians in clinics using a standardised Covid-19 follow-up protocol and in-clinical medical assessments to define PCC, identify risk factors for PCC and recovery rates.

Implications of all the available evidence

In this study, using a rigorous approach and objective medical assessments, we showed a significant, long-lasting impact of Long Covid in children and adolescents, for some children up to 36 months after the initial infection. In addition to providing also several demographic and clinical predictors of Long Covid, we also found an association between initial waves and development of autoimmune diseases following SARS-CoV-2 infection, expanding our knowledge on the link between viral infections and subsequent immunological events. COVID-19 vaccines were associated with a lower risk of developing Long Covid particularly in adolescents, while re-infections had a minimal burden on most patients, although on case of Long Covid following re-infection was identified. Altogether, our findings reinforce the urgent need to fund clinical and research centers to develop diagnostic tests, evaluate if chronic inflammation is involved in pediatric Long Covid, and explore therapeutic strategies. Such achievements will also help to better understand the wider burden of post-acute consequences of viral infection on children on physical health, wellbeing and education.

Introduction

Several independent studies have demonstrated that SARS-CoV-2 infection can cause a long-lasting pattern of debilitating symptoms that are currently affecting millions of people worldwide.[1] This condition, known as Long Covid or Post Covid Condition or Post-Acute Sequelae of SARS-CoV-2, is characterized by the persistence of signs and symptoms that were not present before SARS-CoV-2 infection and lasting at least 12 weeks, negatively impacting daily life.[2]

Long Covid has been widely described both in adults and children, particularly adolescents, and can also follow mild to asymptomatic infections or reinfections.[3] Over the past year, substantial progress has been made in understanding this phenomenon, with several studies shedding light on its underlying mechanisms. Some studies found specific patterns in biological markers consistent with thromboinflammation, persistent immune activation, and dysregulation in adults with Long Covid.[4–6] Conversely, pediatric research has mainly focused on observational studies describing primarily self-reported symptoms proxy-reported parents via phone calls or online surveys, or on large datasets of electronic healthcare records.[7] Both designs have biases since may not adequately refer to the currently accepted definition of Long Covid, which requires that a clinician have ascertained that the persisting symptoms are negatively impacting daily life and have evidence of a prior infection with SARS-CoV-2. In addition, both designs, if not coordinated by physicians experienced with Long Covid, can either overestimate the problem or miss subtle and difficult-to-classify symptoms like brain fog or post-exertional malaise. Our previous study[3] addressed some of these limitations. We conducted a prospective follow-up of children with SARS-CoV-2 infection, assessing participants in person under the guidance of experienced clinicians. We found that not all patients with Long Covid recovered after 18 months post-infection. Risk factors for Long COVID at 3 and 6 months included age (>10 years), comorbidities, hospitalization during the acute phase, and infection with pre-Omicron variants. However, our study primarily focused on the initial six months of follow-up and did not assess re-infections or other complications, such as development of autoimmune conditions, which have been associated with

SARS-CoV-2 infection in adults.[8] So far, only two prospective cohorts in the United Kingdom performed a follow-up study up to 12 months, confirming that Long Covid can last at least a year.[9,10] However, also these studies were based on online surveys.

In this study, we further investigated the longer-term persistence of symptoms and risk factors for developing Long Covid development in a large cohort of children using in-clinic assessments, now assessed up to 36 months after infection. Additionally, we explored whether patients experienced new-onset autoimmune disorders and assessed the impact of re-infections.

Methods

Study population and setting

This is a prospective study conducted at a referral paediatric post-covid clinic in Rome, Italy, on children who were infected with SARS-CoV-2, as we previously described in a preliminary study where we published the first months of follow-up.[3] The study population included children and young people aged up to 18 years of age only. Patients were referred to the public post-COVID outpatient unit by the hospital Emergency Department, Admission Ward, or external family pediatricians (between 01/02/2020 and 28/02/2024). Once referred, the participants were assessed at 3, 6, 12, 18, 24 and 36 months after the initial SARS-CoV-2 infection. All the children were initially assessed in-person by two experienced doctors during the first time-points (3 and 6 months after infection) and subsequently by phone if they reported normal return to pre-covid health (were therefore considered healthy), or in person if they were diagnosed as Long Covid. Importantly, children assessed at each time point were not new children only seen in an isolate visit (eg, first assessment at 12 months but not at 3 and 6 months), but were children that were already evaluated at previous timepoints. Consequently, we did not include children for which a clinical assessment at previous timepoint was not available.

At the time of the visit information on the severity of the acute infection, hospitalization and medication use, symptoms experienced during the acute COVID-19 infection, and the outcome of the acute SARS- CoV-2 infection, Covid-19 vaccination status including number of doses (assessed by evaluating the online vaccination status certificate which was implemented in Italy by the national ministry of health), and demographic data (age, gender, and pre-existing conditions) were collected. The presence and duration of fever, rhinitis, pharyngitis, cough, dyspnea, chest pain, muscle and joint pain, fatigue, headache, rash and skin lesions, gastrointestinal symptoms (diarrhea, vomiting, abdominal pain, loss of appetite, anorexia), weakness, neurological symptoms and other symptoms such as cognitive disorders (sleeping problems, lack of attention, memory loss) were all recorded in detail in the symptom log. Development of new autoimmune disorders, following the initial acute Sars-CoV-2 infection, were also considered. Re-infections that occurred throughout the follow-up time were documented. The data collected included the number of total further COVID-19 infections, state of the child's vaccinations (if they had already received a COVID vaccine or not), whether newly acquired infection was symptomatic or asymptomatic, and symptomatology. Data on the dominant circulating variant at the time of infection was collected from the report coordinated by the Italian Superior Health Institute.[11] We excluded patients aged 19 years or older, those with suspected infections not confirmed by laboratory tests, and those with persistent acute infections.

Outcome definition

Long Covid was defined based on criteria developed by Stephenson et al.[12] and the subsequent WHO post covid-19 condition definition.[2] It encompasses persistence of symptoms for at least three months after the first infection, or new-onset of symptoms that were never reported before the infection, which had a detrimental impact on everyday life, with other probable diagnosis rejected. Tests were performed in every patient who had this potential illness to rule out the following conditions: anemia, hematologic disorders, celiac disease, glycemia, liver and kidney function, thyroid issues and other infections, such as intestinal parasites. This allowed to ensure that individuals with alternate diagnoses were not included in the analysis and were not classified as long-COVID patients. Patients without symptoms, or with minor symptoms that did not interfere with everyday activities, academics, or sports were categorized as "recovered" from the initial infection. Those patients initially diagnosed with Long Covid that, at the following follow-up, reported complete recovery and disappearance of the persisting symptoms and return to normal daily activities, were classified as "recovered from long covid" and considered healthy children.

Statistics

Descriptive data are shown as absolute numbers and percentages for categorical variables, and median and interquartile range (IQR) for continuous ones. Normality of data was assessed with Kolmogov–Smirnov test. At each follow-up time point, the number of patients is described in Fig. 1, including those eligible for that timepoint assessment, those actually seen and those that missed the appointment. We specified this information because patients were enrolled during a wide time period and most cases were infected during the omicron wave, therefore may have not reached yet the 24 or 36 timepoints. Baseline differences between patients who were lost to follow up and those who presented at each follow up are reported in Supplementary Material. Missing data were addressed using Inverse

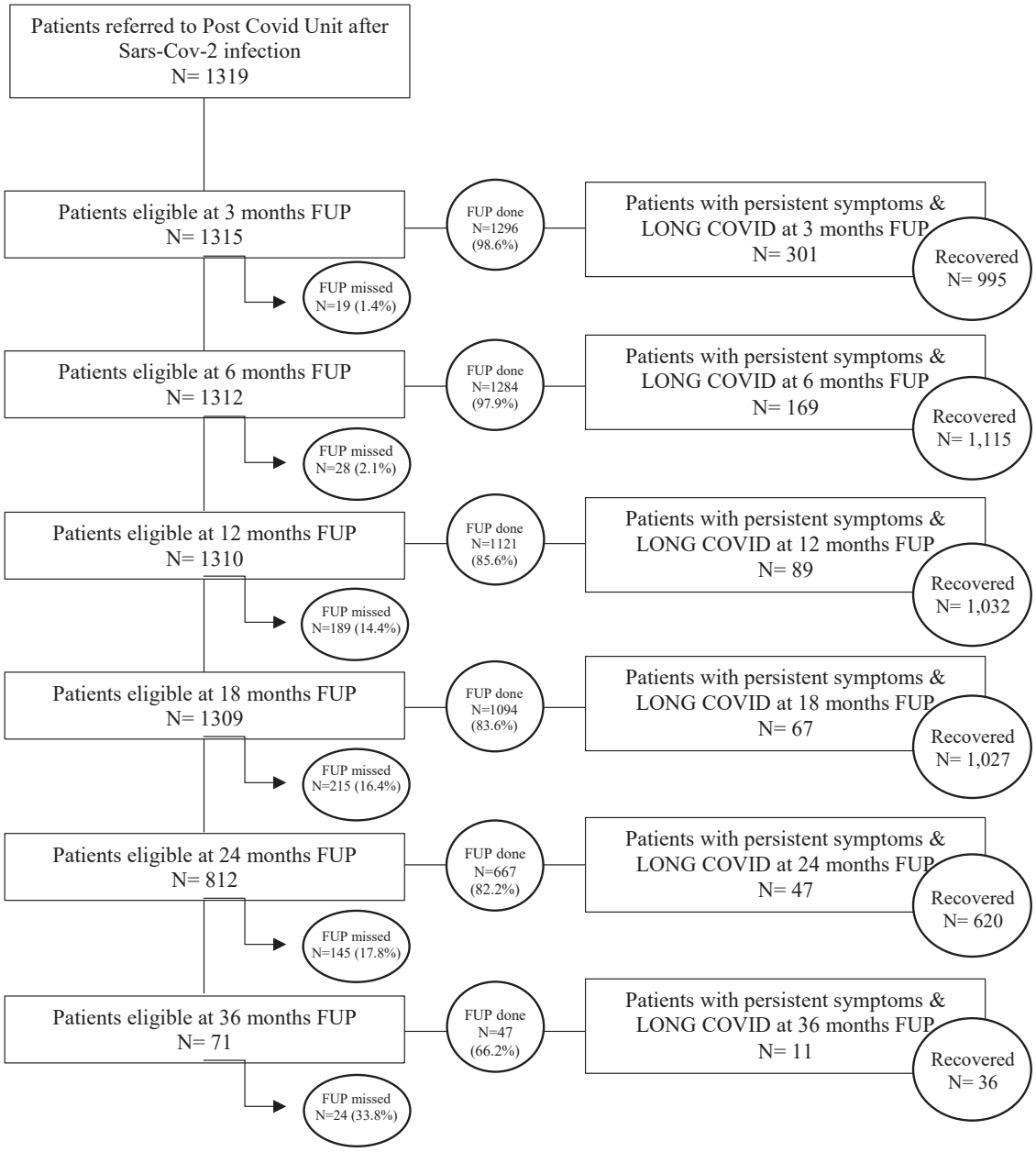

Fig. 1: Patients flow diagram and number of children with persisting symptoms (LONG COVID) development at each follow-up evaluation.

Probability Weighting (IPW), where weights were calculated based on the probability of being observed, estimated from a logistic regression model including age, sex, comorbidities, Covid severity at first infection, need for hospitalization.[13]

For the outcomes "presence of Long Covid" at 3-6-12 months, logistic regression models have been used to study the effect of age, sex, vaccination, different Covid variants. Goodness of fit of the models was assessed with Hosmer-Lemeshow test. Possible final models were evaluated comparing their respective Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) where indicated.

Since different Covid variants had different correlations with LC at different timepoints, a subsequent analysis was carried out separately for the groups of children, differentiated by age in relation to differing vaccination eligibility (5–11 years and 12–18 years), at three-, six-, twelve-, and eighteen-month follow-ups. The 11 years cut-off was only used for the vaccination

analyses, and was based on the eligibility of different cohorts for covid vaccines in children (5–11 yrs and 12–18 yrs, which also received different doses of vaccination but also accessed to vaccination in different periods). Each age category and temporal outcome was studied with a multilevel mixed logistic model with random effects on the Covid variant unless the respective Likelihood Ratio was not significant. In this case, a logistic regression model was used.

The persistence of different symptoms at different timepoints was studied longitudinally up to 12 months with a generalized multilevel mixed model logistic regression for correlated outcomes, conditional on the Covid variant and including vaccination, comorbidities, and age category as covariates.

This is a descriptive analysis without any specific *a priori* hypothesis; we did not pre-specify a sample size and have included all patients who presented to the clinic in the given period and signed the informed consent. This was due to the lack of knowledge on the incidence of Long Covid in children when we started our study, which is still unclear. All statistical tests were two-sided, and the level of statistical significance was set at 0.05. Reported P-values have not been corrected for multiple hypothesis testing. Data were analyzed with Stata v. 18 BE (Statacorp, LLC, Texas, USA).

Ethics

Informed permission was given, and the study was authorized by the Ethics Committee of the Fondazione Policlinico Universitario A. Gemelli IRCCS of Rome, Italy (Ethic approval ID4518, Prot0040139/21). As per the local guidelines of the ethical committee, ethical consent was obtained from parents/caregivers and children who were older than 5 years old, with dedicated consent adapted for age.

Role of the funding source

The study was funded by Pfizer to Dr Danilo Buonsenso. The project's creation, study design, data collecting, analysis, result interpretation, and report writing were all outside the purview of the donors. DB made the ultimate choice to submit the dataset for publication and has complete access to it. Each author accepted responsibility for the decision to submit the work for publication and had complete access to all study data.

Results

Study population

A total of 1319 patients were included (Fig. 1), the median age was 87 (48–124) months, 45.9% were females (Table 1), and 14.4% (190) had pre-existing comorbidities (Supplementary Material). During acute infection, 8.6% (114) were asymptomatic, 88.6% (1169) had mild, 2.2% (29) moderate and 0.2% (2) severe COVID-19. Most children were diagnosed with COVID-19 when

	Total
	N = 1.319
Demographics	
Female sex, n (%)	604 (45.9)
Age (months)	87.0 (48.0–124.0)
Age category	
0–4 years n (%)	334 (25.3)
5–12 years n (%)	712 (54)
>12 years n (%)	273 (20.7)
Comorbidities n (%)	190 (14.4)
Severity of acute SARS-CoV-2 infection	
Asymptomatic n (%)	115 (8.8)
Mild n (%)	1169 (88.6)
Moderate n (%)	29 (2.2)
Severe n (%)	2 (0.2)
Missing n (%)	4 (0.4)
COVID-19 variant	
Original n (%)	43 (3.3)
Alfa n (%)	79 (6.0)
Delta n (%)	252 (19.1)
Omicron n (%)	939 (71.2)
Missing n (%)	6 (0.5)
COVID-19 vaccine doses, n (%)	
0	1049 (79.6)
1	82 (6.2)
2	166 (12.6)
3	20 (1.52)
Unknown	2 (0.2)
Symptoms during acute infection	
Fever	
No n (%)	432 (32.8)
Yes n (%)	880 (66.7)
Missing n (%)	7 (0.5)
Rhinitis n (%)	603 (45.9)
Cough n (%)	483 (36.8)
Fatigue n (%)	293 (22.3)
Headache n (%)	291 (22.2)
Gastrointestinal symptoms n (%)	187 (14.2)
Muscular pain n (%)	116 (8.8)
Pharyngodynia n (%)	93 (7.1)
Joint pain n (%)	85 (6.5)
Anosmia n (%)	66 (5.0)
Disgeusia n (%)	58 (4.4)
Chest pain n (%)	36 (2.7)
Hospitalization during acute infection n (%)	30 (2.3)
Rash n (%)	24 (1.8)
Rest dyspnea n (%)	24 (1.8)
Exertion dyspnea n (%)	22 (1.7)
Asthma n (%)	13 (1.0)
Myocarditis during acute infection n (%)	3 (0.02)
Oxygen supplementation n (%)	1 (0.1)
NIV n (%)	1 (0.1)
Fever days	1.0 (0.0–2.0)
Pediatric Intensive Care Unit admission during acute infection n (%)	1 (0.1)

(Table 1 continues on next page)

	Total
	N = 1.319
(Continued from previous page)	
CPAP n (%)	0 (0)
Respiratory failure n (%)	0 (0)
Invasive Ventilation	0 (0)
Therapies	
Steroids during acute infection n (%)	23 (1.8)
Remdesivir during acute infection n (%)	1 (0.1)
IVIG during acute infection n (%)	1 (0.1)
Antibiotics during acute infection n (%)	38 (2.9)

Table 1: **Clinical and demographic characteristics of the study population. Data are expressed as N (%) or median (IQR).**

the Omicron variant was prevalent (939, 71.2%). 79.6% (1049) children were not vaccinated before the infection, while respectively 6.2% (82), 12.6% (166), and 5.2% (20) children had received one, two, and three doses of COVID-19 vaccine. Vaccination status according to Long Covid status at three months is reported in Fig. 2.

Symptoms during acute phase and follow-up

Fever (66.7%), rhinitis (45.9%), cough (36.8%), fatigue (22.3%), and headache (22.2%), were the most commonly reported symptoms during the acute phase (Table 1).

Among the 1296 patients who underwent a 3-month follow-up assessment, 23.2% (301) reported persistent or newly developed symptoms with a negative impact on daily activities, meeting the criteria for Long Covid. 169 (13.2%) patients remained symptomatic at six months, 89 (7.9%) at 12 months, 67 (6.1%) at 18 months, 47 (7.1%) at 24 months (Table 2). Only 47 children reached the 36 months milestone, of them 11 (23.4%) having persisting symptoms. Five (0.4%) children were diagnosed with Multisystem Inflammatory Syndrome (MIS-C). The most reported persisting symptoms at the first follow-up at three months after infection were fatigue (13.1%), exertional dyspnea (6.3%), headache (5.9%), and gastrointestinal symptoms (4.9%) (Table 2).

Factors associated with a diagnosis of long covid lasting three months or more

For the primary outcome of Long Covid at three, six, twelve and eighteen months, multivariable logistic regression analysis models were performed for each timepoint including age, vaccination, SARS-CoV-2 variant, female sex and presence of comorbidities. Older age, comorbidities, infection during original and alfa variants and female sex were predictors of Long Covid at three months, and most of these factors with Long Covid at 6, 12 and 18 months follow-up (Table 3). In particular, we found 12 years of age as having a steep increase in the risk of Long Covid. We have also performed a multivariable logistic regression analysis on the outcome Long Covid at 3-6-12-18 months in the only non vaccinated population, finding similar results. In particular, infection with pre-omicron variants remained

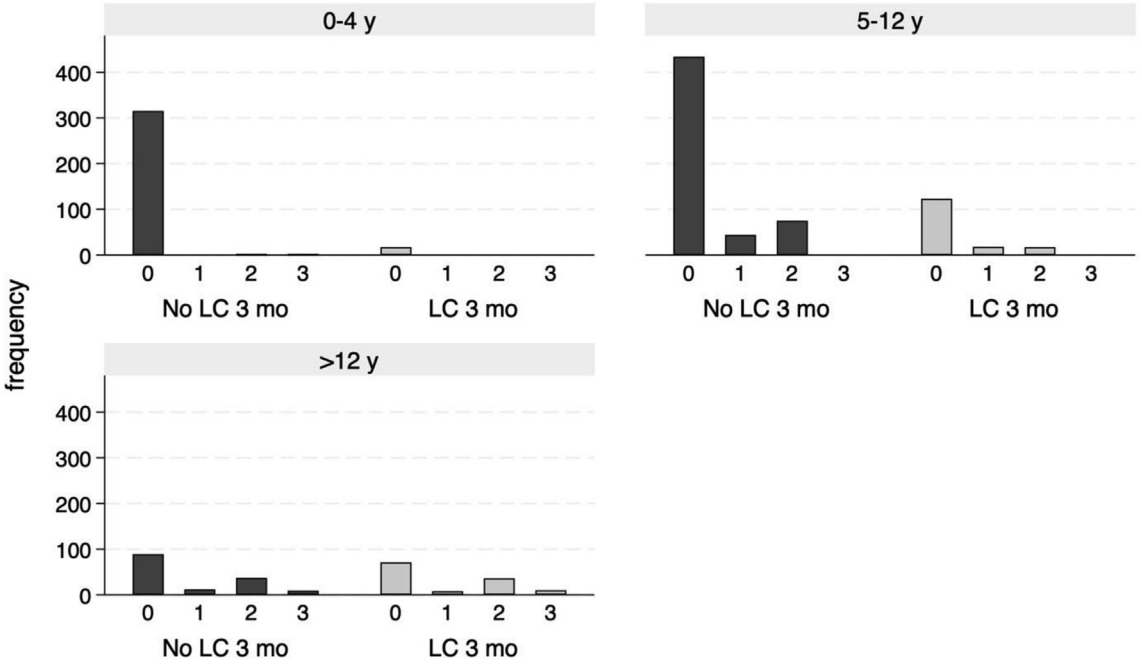

Fig. 2: Interactive effect of number of vaccine doses and age on the outcome Long Covid at different time points.

	All patients					Not vaccinated				
	3 mo	6 mo	12 mo	18 mo	24 mo	3 mo	6 mo	12 mo	18 mo	24 mo
Children that reached the specific timepoint follow-up	1296 patients	1284	1121	1094	667	1029	1019	883	863	572
Long Covid n (%)	301 (23.2)	169 (13.2)	89 (7.9)	67 (6.1)	47 (7.1)	207 (20.1)	121 (11.9)	65 (7.3)	49 (5.7)	37 (6.3)
Recovered n (%)	995 (76.7)	1115 (86.8)	1032 (92.1)	1027 (93.8)	620 (92.9)	822 (79.9)	898 (88.1)	822 (92.7)	814 (94.3)	536 (93.7)
MIS-C n (%)	5 (0.4)	/	/	/	/	/	/	/	/	/
Persisting symptoms										
Any Symptoms n (%)	301 (23.2)	164 (12.8)	98 (8.7)	78 (7.1)	50 (7.5)	210 (20.4)	123 (12.07)	73 (8.2)	59 (6.8)	40 (6.9)
Fatigue n (%)	171 (13.1)	83 (6.5)	43 (3.8)	28 (2.6)	24 (3.6)	120 (11.5)	60 (5.9)	33 (3.7)	20 (2.3)	18 (3.1)
Post Exercise Dyspnea n (%)	82 (6.3)	44 (3.4)	22 (2.0)	13 (1.2)	10 (1.5)	50 (4.8)	29 (2.9)	15 (1.7)	8 (0.9)	7 (1.2)
Headache n (%)	77 (5.9)	34 (2.6)	23 (2.1)	20 (1.8)	13 (1.9)	53 (5.1)	24 (2.3)	16 (1.8)	14 (1.6)	11 (1.9)
GI Symptoms n (%)	63 (4.9)	31 (2.4)	10 (0.9)	8 (0.7)	6 (0.9)	51 (5)	28 (2.7)	9 (1)	7 (0.8)	5 (0.8)
Muscle Pain n (%)	58 (4.5)	26 (2.0)	17 (1.5)	7 (0.6)	5 (0.7)	44 (4.2)	23 (2.2)	15 (1.7)	7 (0.8)	5 (0.8)
Joint Pain n (%)	36 (2.8)	17 (1.3)	13 (1.2)	6 (0.5)	4 (0.6)	28 (2.7)	17 (1.6)	11 (1.2)	6 (0.7)	4 (0.7)
Chest Pain n (%)	33 (2.5)	19 (1.5)	11 (1.0)	5 (0.5)	3 (0.4)	24 (2.3)	15 (1.4)	9 (1)	3 (0.3)	2 (0.3)
Tachicardia n (%)	21 (1.6)	13 (1.0)	11 (1.0)	11 (1.0)	7 (1.0)	9 (0.9)	6 (0.6)	5 (0.6)	6 (0.7)	3 (0.5)
Cough n (%)	15 (1.2)	8 (0.6)	16 (1.4)	18 (1.6)	6 (0.9)	12 (1.1)	6 (0.6)	13 (1.4)	15 (1.7)	6 (1.0)
Rash n (%)	14 (1.1)	6 (0.5)	6 (0.5)	5 (0.5)	5 (0.7)	11 (1)	5 (0.5)	3 (0.3)	3 (0.3)	4 (0.7)
Fever n (%)	12 (0.9)	3 (0.2)	2 (0.2)	2 (0.)	1 (0.1)	7 (0.7)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)
Anosmia n (%)	12 (0.9)	8 (0.6)	3 (0.3)	2 (0.2)	2 (0.3)	9 (0.9)	7 (0.7)	2 (0.2)	2 (0.2)	2 (0.3)
Disgeusia n (%)	12 (0.9)	8 (0.6)	3 (0.3)	1 (0.1)	1 (0.1)	9 (0.9)	7 (0.7)	2 (0.2)	1 (0.1)	1 (0.1)
Asthma n (%)	11 (0.8)	8 (0.6)	9 (0.8)	9 (0.8)	5 (0.7)	7 (0.7)	5 (0.5)	7 (0.7)	8 (0.9)	4 (0.7)
Rest Dyspnea n (%)	6 (0.5)	3 (0.2)	2 (0.2)	2 (0.2)	2 (0.3)	2 (0.1)	2 (0.2)	0 (0)	0 (0)	1 (0.1)

Table 2: **Outcomes and persisting symptoms at different follow-up times. Data are expressed as N (%).**

a statistically significant risk factor for the development of Long Covid (Supplementary Material, Table 6Sa).

While vaccination did not prove an overall protective effect towards LC by itself, its role was further analyzed across the different age categories, across the number of shots received and across the different SARS-CoV-2 variants. We therefore used a multilevel mixed logistic model, with random effect on the SARS-CoV-2 variant. Numerical results are reported in the Supplementary Material.

For children aged 0–4 years, as virtually no patient had been vaccinated (see Fig. 2), no analysis was possible.

For children aged 5–11 years, a significant effect of three doses of vaccine was noted on presence of Long Covid at 3 months in the sense of reduction (see Fig. 3; Table 22S in Supplementary Material). Considering LC at 6 months, a significant effect of three vaccine doses compared to no vaccination was present for in terms of reduction of probability to develop LC (Table 24S; Fig. 3). At 12 months, three vaccine doses were significant for reduction of LC (Table 26S).

For children aged 12 years and above, one vaccine dose had a significant effect on reduction probability of LC at 3 months from the age of 14 upwards (Table 23S; Fig. 3).

At 6 months, one dose of vaccine was significant in reduction of LC and from the age 13 upwards and 3 doses of vaccine had a significant reductive effect at ages 12–14 (Table 25S; Fig. 3). At 12 months, three doses of

	3 months			6 months			12 months			18 months		
	OR	P > z	[95% CI]	OR	P > z	[95% CI]	OR	P > z	[95% CI]	OR	P > z	[95% CI]
Vaccine	1.34	0.11	0.93–1.93	1.51	0.184	0.81–2.78	1.62	0.21	0.76–3.44	1.35	0.476	0.58–3.11
Age category												
5-11 y	4.69	<0.001	2.63–8.38	4.44	<0.001	2.20–9.43	2.84	0.024	1.14–7	1.1	0.809	0.48–2.53
>12 y	7.98	<0.001	4.2–15.15	11.33	<0.001	5.21–24.63	7.31	<0.001	3–19	3.7	0.002	1.58–8.64
Comorbidity	1.61	0.025	1.06–2.44	1.83	0.008	1.17–2.88	1.74	0.062	0.97–3.13	1.15	0.693	0.56–2.33
Original variant	5.85	<0.001	2.46–13.47	4.77	<0.001	2.23–10.22	4.58	0.001	1.92–10.92	3.52	0.012	1.32–9.37
Alfa variant	5.72	<0.001	3.04–10.78	8.18	<0.001	4.60–14.56	7.61	<0.001	4.08–14.18	4.09	<0.001	2.01–8.3
Female sex	1.39	<0.001	1.02–1.89	1.62	0.008	1.13–2.32	1.54	0.072	0.96–2.47	1.08	0.748	0.64–1.83

Table 3: **Multivariable logistic regression results on the outcomes "Long Covid at 3-6-12-18 months".**

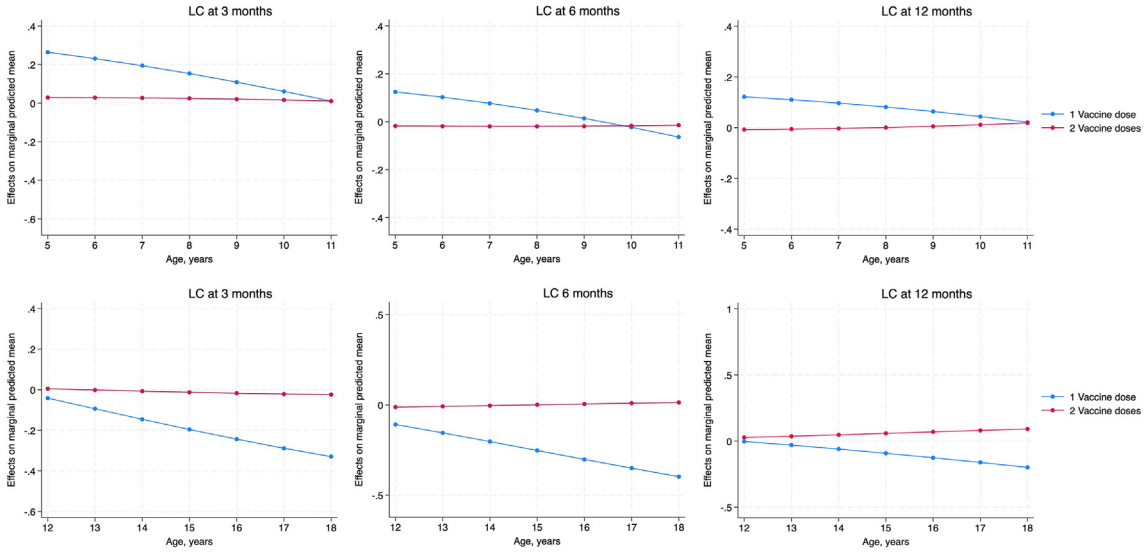

Fig. 3: Interactive effect of number of vaccine doses and age on the outcome Long Covid at different time points. Asterisks show significance. Relative significance and CI are reported in Supplementary Material.

vaccine presented a significantly difference of effect with a reduction of probability until the age of 16, then an effect in the sense of more LC for ages 17–18 (Table 27S).

The Supplementary Material shows the multivariate logistic regression models for the prediction of the commonest persisting symptoms at the different follow-up timepoints. Age, hospitalization, days of fever and pre-omicron variants at the time of acute infection were risk factors for several persisting symptoms at different timepoints. Use of steroids during acute infection were associated with persisting muscle pains and post-exertional malaise at three and six months following the initial infection.

Effect of vaccination on symptoms' persistence at different timepoints

A multilevel mixed effects logistic regression model for correlated outcomes was applied to presence of different symptoms at different timepoints in order to study the effect of vaccination, comorbidities and age category on the persistence of specific symptom, conditional on SARS-CoV-2 variant. Vaccination was particularly protective in preventing persistence of gastrointestinal symptoms (Supplementary Material).

New infections

Data about re-infections were obtained from 1125 patients. Overall, 214 (19%) had one and 29 (2.58%) two documented re-infections. None of them needed to be admitted. One patient was diagnosed with Long Covid after a re-infection.

Having comorbidities, being infected with pre-omicron variants and having a diagnosis of Long Covid at three months follow-up were significantly associated with a higher risk of re-infections. However, only an initial asymptomatic infection was significantly associated with a symptomatic re-infection (Supplementary Material).

The probability of a new infection was studied according to age of the patient and number of vaccine doses received. It showed no significant effect of one dose of vaccine compared to no vaccine, but a significant reduction in probability of a new infection for 2 doses of vaccine at any age and a significant reduction of probability for 3 doses of vaccine after the age of 14 years (Supplementary Material).

New-onset autoimmune disorders

15 patients (1.1%) developed autoimmune disorders. In particular, two patients developed Hashimoto Thyroiditis, four developed Celiac Disease, one developed Systemic Lupus Erythematosus, three developed Chronic Autoimmune Urticaria, and the other five undifferentiated autoimmune connective tissue disease. Being infected with the original SARS-CoV-2 variant was associated with a higher risk of developing autoimmune disorders (P = 0.035, 95% CI 1.12; 2.4).

Discussion

To our knowledge, this is the largest and longest prospective follow-up study of children post SARS-CoV-2 infection, assessed in clinical settings using a standardized Long Covid definition and protocol. In this study, we further confirmed that children can have a significant burden of long-lasting persisting symptoms after SARS-CoV-2 infection, and although most children

recover over time, some are still symptomatic 36 months following the first infection.

In line with our previous study and others, the most commonly persisting symptoms identified were fatigue, post-exertional dyspnea, headache, and gastrointestinal symptoms. Long Covid remained more frequent in older children, those with specific pre-existing comorbidities and initially hospitalized, those infected with during pre-omicron waves.[3,14] In addition, we found that specific characteristics at time of the initial infection are associated with a higher risk of developing Long Covid lasting three or more months, including headache, fatigue, duration of initial symptoms. These association, which has never been published before in pediatric studies, may have implication as these patients may deserve a stricter follow-up, or may benefit to enter trials for early preventive treatment of Long Covid, if established in future. Specific clinical symptoms during acute infection were associated with persistence of symptoms up to 24 months, like duration of fever. Interestingly, use of steroids during acute infection were associated with persistent muscular symptoms at different time points. Although it is known that corticosteroids can induced myopathy, this mostly occurs as an adverse effect of prolonged oral use,[15] so possibly other speculative mechanisms can play a role in this case. In any case, as steroids should not be used during acute mild COVID-19 infections, this association we found further reinforce the importance of avoiding non-evidence based therapies for pediatric COVID-19.

The observation of persisting symptoms for up to 36 months in some patients is worryingly. So far, pediatric Long Covid studies have mostly focused on 6–12 months follow-up, showing that most children recover. While it is true, this does not happen to all, as also extensively reported in adults,[16–21] posing a significant burden not only for the affected patients, but for their families as well. Several family associations globally are in fact reporting a significant economic and routine burden for parents of children severely affected by Long Covid (https://www.longcovidkids.org/support-services). The demonstration of objective physical limitations and other organ damage in children and adolescents with Long Covid further reinforce such a burden. Of note, in fact, the same children with Long Covid that we followed in this study are the same that have been enrolled in other diagnostic studies were we documented abnormal lung perfusion,[22,23] brain metabolism,[24,25] autonomic dysfunction,[26] cardiorespiratory and muscle impairments,[27] circulating activated platelets[28] and pro-inflammatory signatures (paper under review). Possible mechanisms leading to such persisting symptoms and organ damage may be related to persistent SARS-CoV-2 infection in reservoirs,[29,30] chronic immune activation,[31,32] thromboinflammation,[4] or development of autoantibodies,[33] or a mix of these mechanisms.[34] Of note, although some of our patients were lost to follow-up, particularly at the last planned visits, it is highly probable that these patients were fully recovered and decided not to respond to latest assessments, as a drop-out of patients with Long Covid would not be expected as no other similar pediatric centers are yet established in our country.

As such, our study urges significant efforts from funding agencies and policy makers to establish clinical and research centers for pediatric Long Covid that, ideally, have similar pathways, measure the same outcomes,[35] and collaborate each other.[36] This should remain valid even if we found that children infected with during the latest "COVID-19 waves" had a significantly lower risk of developing Long Covid compared with those infected with the first waves. While this reduced risk is consistent with other pediatric and adult studies,[3,14,37–41] Long Covid in those infected since omicron waves is still a non-rare possibility, as reported by our cohort and others, given the high viral circulation.[9,42]

In this updated follow-up, we have further assessed the role of COVID-19 vaccines in preventing the risk of Long Covid in children. Given the nature of a multiple follow-up studies, we could assess their roles not only in preventing the development of Long Covid at three months, but at different time points up to 18 months follow-up. To our knowledge, this is the first time such a data has been made available. In our previous study, in fact, children with two doses of vaccination had a lower, although not statistically significant, risk of Long Covid.[3] Messiah et al. showed that patients who did not report vaccination information were six-times more likely to develop Long Covid (RR: 5.76, 95% CI: 1.18–28.06).[38] In a recent, larger US cohort presented, COVID-19 mRNA vaccination in children was moderate protective effect of against Long Covid, stronger in adolescents, who have higher risk of long Covid, and waned over time.[43] These data however, were obtained through electronic health records. Given the particular cohort of our study, we cannot draw inference on the general pediatric population. Our results, however, are in line with the US dataset and show that those who were vaccinated had a lower probability of Long Covid at different time points, also considering the variability of the Covid variant during the different periods. Interestingly, we found an age-stratified benefit for specific age groups, which may help informing families about personalized risk-benefit ratios of vaccination. Of note, our data were corrected for multiple factors including age, comorbidities and Covid variants. The paradox increase in Long Covid rates in older adolescents aged 17–18 years old vaccinated with three doses may be due to a selection bias. In fact, only a minority of the whole study population did three vaccinations, while our unit see more patients with Long Covid, particularly among older adolescents which are at higher risk of developing it, being one of the only pediatric centers in the countries. Therefore, the few that developed Long Covid despite three

vaccinations may have averted the statistical results, since the denominator of the whole population with three doses was very low (only 20 out of 1317 children received three doses). In any case, also all adult studies have provided exactly similar findings of reduced risk of Long Covid in vaccinated population,[44] making the pediatric data reasonable, although not perfect.

A limited number of studies have evaluated SARS-CoV-2 re-infections in children. In our cohort, microbiologically confirmed re-infections were not rare, since 214 (19%) had one and 29 (2.58%) two documented re-infections. None of these children had a severe infection, confirming previous studies that both natural and vaccine immunity are protective against severe disease in children.[45] Differently from other studies, in our cohort significant reduction in probability of a new infection for 2 doses of vaccine at any age and a significant reduction of probability for 3 doses of vaccine after the age of 14 years, suggesting that boosters do have a role in reducing the risk of reinfections. These findings are in line with the few available from older studies. In a national surveillance study to assess reinfection of SARS-CoV-2 in children in England at least 90 days after primary infection from Jan 27, 2020, to July, 31, 2021, the pediatric reinfection rate was reinfection rate was 0.68% and were not associated with more severe disease or fatal outcomes.[46] In Kuwait, during a similar timepoint, re-infections were uncommon, and most children (55.2%) had asymptomatic reinfection.[47] Similarly, in Serbia and Turkey pediatric reinfections were rare and milder.[48,49]

Interestingly, in our cohort one child (1/249, 0.4%) developed Long Covid after a second infection contracted during the omicron wave in Italy. This finding is relevant as Long Covid may be, nowadays when COVID-19 is usually mild in children and MIS-C significantly rare,[50] the most worryingly complication. Our study is in line with a UK Prospective Observational Study that showed that children re-infected during Omicron can still develop Long Covid.[9] Of note, having Long Covid was significantly associated to a higher risk of re-infections. Although this may be biased by the fact that chronically ill patients may be more prone to test themselves or seek medical care in case of covid-like symptoms, it is also plausible that children with Long Covid are more susceptible to have recurrent symptomatic viral infections, as already suggested in adults with Long Covid[51] or ME/CFS.[52] These findings highlight the need of continuing the research to understand the causes and treatments of Long Covid both in adults and children.

We assessed how many children in our cohort developed autoimmune disorders. The reasons we looked for this outcome was the association, found in several adult cohorts, that people who were infected with SARS-CoV-2 had a substantially increased risk of developing a diverse spectrum of new-onset autoimmune diseases.[8,53,54] About the pediatric population, an increase in new diagnoses of type 1 diabetes since the beginning of the pandemic has been reported, but the direct association with SARS-CoV-2 infection is still debated.[55] Other small case reports or case series have shown new onset autoimmune diseases following SARS-CoV-2 infection in children.[56,57] Our study, to our knowledge, has been the first that have evaluated new diagnoses of these disorders in a large cohort of children prospectively followed-up after Covid-19. Surprisingly, we found a relatively high proportion of new diagnosis (1.1%, 15 cases), and multivariate analyses found a significant association with the original SARS-CoV-2 variant. The 1.1% prevalence seems particularly high, considering that current prevalence of autoimmune diseases in children seem to be around 0.4%.[58–60] These findings would be in line with other reports showing that SARS-CoV-2 infection may trigger the development of auto-antibodies[61] and further expand to the growing body of literature linking our knowledge about viral infections and autoimmunity, including recent discoveries about Epstein-Barr virus and multiple sclerosis.

The findings from our study further reinforce our knowledge on the global impact of Long Covid on humans, adding to available evidence from the adult population. Many of our findings, in fact, are in line with adult studies, including a higher risk of developing Long Covid in adults infected with the original variant,[62] the lower risk in those previously vaccinated,[63] and the long-lasting duration of persisting symptoms in many affected patients.[64] In adults, early antiviral treatment was also associated with a lower risk of developing Long Covid, however we could not assess this point as antivirals for mild pediatric infections are not used in our country.[65]

Public health agencies should be aware of our findings making long-term complications of new infections as a top priority to be addressed, both in terms of communication with the public opinion, and in terms of allocating appropriate resources for immediate assessment. Most of children suffering from Long Covid are labelled as having a functional disorder, despite the major immunological findings behind this syndrome has been demonstrated in adults, and our study demonstrate clinical similarities between adult and pediatric Long Covid. Our data can also inform public health communication about potential risk of developing Long Covid in case of new infection, particularly with a society that is mostly not updating vaccination coverage. In fact, a recent, large population study from the United States has also found a significant role of vaccination in reducing the risk of Long Covid in children.[66]

Our study is not without limitations. First, this is a single-center study, although all children with a positive Sars-CoV-2 infection were referred to participate from outpatient family pediatricians and not only from our

Institution. Secondly, we did not have access to sequencing of the variants at the time of diagnosis, and these data were estimated from reports on the most prevalence variant circulating in the city at the time. In addition, we were not able to rule out any preceding asymptomatic SARS-CoV-2 infection in children whose symptomatic infection was diagnosed during delta and omicron waves, although it is known that most children have been infected during Omicron, and that multiple reinfections were rare with previous variants.[67] Also, the incidence of Long Covid observed in our cohort may be inflated due to our clinic being one of the few pediatric Long Covid clinics in the country, resulting in a higher proportion of patients with persistent symptoms seeking care at our center. However, understanding the prevalence of Long Covid was not an aim of our study. While the missing data at the 6-month follow-up may not fully satisfy the Missing at Random (MAR) assumption, our analysis using Inverse Probability Weighting yielded strongly significant results. The low percentage of missing data further mitigates any potential bias. Future research, particularly randomized trials on treatments, could explore methods more robust to departures from MAR. We have not discussed treatments used in our children. As previously published, our diagnostic protocol for Long Covid is personalized and based on main symptoms, and as such is our treatment (eg. chronic headache is managed according to current guidance for headache, and so on), since there is so far no formal treatment). Last, we have not measured hormonal levels nor Tanner stages in our cohort, this would have been interesting as Long Covid was higher in teenagers and we might speculate the role of hormones on immunity, however we did not predict this finding when we developed our study. Despite these limitations, one of the important strenghts of our paper is represented by the use of in-clinic specialist assessments (although clinicians where unblinded) performed at multiple timepoints and based on a rigorous, paediatric clinical case definition of Long Covid identifying children with persistent symptoms that are so severe that they impact on daily activities (although our study began before the WHO release of a pediatric PCC definition, the one we used is perfectly in line with the WHO one released in 2023).[2] Another strength is characterized by the geographical representativeness of our cohort. While Long Covid prevalence can be inflated due to a bias selection, patients included in our study well represent the local population, being a large sample with mostly Caucasian children (as representing the catchup area of our study), and patients were sent by several local settings (our emergency department, our admission ward, and family pediatricians working in the catch up are of our hospital).

In conclusion, using a rigorous definition and a long follow up time including multiple timepoints, we showed a significant, long-lasting impact of Long Covid in children and adolescents, for some up to 36 months after the initial infection. In addition to providing also several demographic and clinical predictors of Long Covid, we also found an association between initial waves and development of autoimmune diseases following SARS-CoV-2 infection, expanding our knowledge on the link between viral infections and subsequent immunological events. COVID-19 vaccines were associated with a lower risk of developing Long Covid particularly in adolescents, while re-infections had a minimal burden on most patients, although on case of Long Covid following re-infection was identified. Altogether, our findings reinforce the urgent need to fund clinical and research centers to develop diagnostic tests, evaluate if chronic inflammation is involved in pediatric Long Covid, and explore therapeutic strategies. Such achievements will also help to better understand the wider burden of post-acute consequences of viral infection on children on physical health, wellbeing and education.

Contributors

DB and DM conceptualised the study. AC and FV were responsible for statistical analyses. DB, RM, ALR, GZ, FR, PV were responsible for patient management and data collection. DM contributed to the protocol development as international advisors, contributed to the first draft of the manuscript and English editing. PV and GZ were responsible for study and team supervision. DB, DM, AC and ALR wrote the initial draft of the manuscript, final version and coordinated the revision process. All authors read and approved final version of the manuscript. DB has full access to dataset and had final decision to submit for publication. DB and AC verified the data. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Data sharing statement

Data collected for the study, including individual participant data and a data dictionary defining each field in the set, will be made available to others after reasonable request to the corresponding author, including the statistical analysis plan and informed consent form. Data will be available as soon the paper is published and shared privately by email. Data will be available for subanalyses, new analyses, confirmatory analyses, with or without investigator support, after approval of a proposal, with a signed data access agreement, for research purposes.

Declaration of interests

DB was been granted a non-competitive grant from Pfizer to study Long Covid in children, and has won a grant to study mRNA profile in children with Long Covid from Roche Italia and ESPID. DB has participated in educational peer-to-peer programs on Long Covid organized by Pfizer and has participated as invited speaker and a sponsored session of COVID-19 vaccines in children at the ESPID conference in 2022.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102815.

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