

Increased SARS-CoV-2 reactive low avidity T cells producing inflammatory cytokines in pediatric post-acute COVID-19 sequelae (PASC)

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Abstract

Background: A proportion of the convalescent SARS-CoV-2 pediatric population presents nonspecific symptoms, mental health problems and a reduction in quality of life similar to myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and long COVID-19 symptomatic. However, data regarding its clinical manifestation and immune mechanisms are currently scarce. **Methods:** In this study, we perform a comprehensive clinical and immunological profiling of 17 convalescent COVID-19 children with post-acute COVID-19 sequelae (PASC) manifestation and 13 convalescent children without PASC manifestation. A detailed medical history, blood and instrumental tests and physical examination were obtained from all patients. SARS-CoV-2 reactive T cell response was analyzed via multiparametric flowcytometry and the humoral immunity was addressed via pseudovirus neutralization and ELISA assay. **Results:** The most common PASC symptoms were shortness of breath/exercise intolerance, paresthesia, smell/taste disturbance, chest pain, dyspnea, headache and lack of concentration. Blood count and clinical chemistry showed no statistical differences among the study groups. We detected higher frequencies of spike (S) reactive CD4+ and CD8+ T cells among the PASC study group, characterized by TNF α and IFN γ production and low functional avidity. CRP levels are positively correlated with IFN γ producing reactive CD8+ T cells. **Conclusions:** Our data might indicate a possible involvement of a persistent cellular inflammatory response triggered by SARS-CoV-2 in the development of the observed sequelae in pediatric PASC. These results may have implications on future therapeutic and prevention strategies.

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All authors declare no competing interests.

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Abstract

Background: A proportion of the convalescent SARS-CoV-2 pediatric population presents nonspecific symptoms, mental health problems and a reduction in quality of life similar to myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and long COVID-19 symptomatic. However, data regarding its clinical manifestation and immune mechanisms are currently scarce.

Methods: In this study, we perform a comprehensive clinical and immunological profiling of 17 convalescent COVID-19 children with post-acute COVID-19 sequelae (PASC) manifestation and 13 convalescent children without PASC manifestation. A detailed medical history, blood and instrumental tests and physical examination were obtained from all patients. SARS-CoV-2 reactive T cell response was analyzed via multiparametric flowcytometry and the humoral immunity was addressed via pseudovirus neutralization and ELISA assay.

Results: The most common PASC symptoms were shortness of breath/exercise intolerance, paresthesia, smell/taste disturbance, chest pain, dyspnea, headache and lack of concentration. Blood count and clinical chemistry showed no statistical differences among the study groups. We detected higher frequencies of spike (S) reactive CD4+ and CD8+ T cells among the PASC study group, characterized by TNF α and IFN γ production and low functional avidity. CRP levels are positively correlated with IFN γ producing reactive CD8+ T cells.

Conclusions: Our data might indicate a possible involvement of a persistent cellular inflammatory response triggered by SARS-CoV-2 in the development of the observed sequelae in pediatric PASC. These results may have implications on future therapeutic and prevention strategies.

Keywords: pediatric PASC, long COVID, T cells, neutralizing antibodies

Main text

Introduction

Already from the first pandemic months, it became evident that clinical manifestations of SARS-COV-2 infection in pediatric patients were strongly differentiated compared to adults(1). Very few children progress to any significant respiratory distress(2,3), however a small percentage of pediatric patients presents multisystem inflammatory syndrome(4,5) as a post infectious complication. Similar to adults, a part of the convalescent COVID-19 pediatric population presents multisystemic nonspecific symptoms, mental health problems and a reduction in quality of life similar to ME/CFS and long COVID-19 symptomatic(6). However, current reports are conflicting regarding its direct or indirect connection to SARS-CoV-2 prevalence, duration and impact on daily life(1). Sorg et al and Borch et al report ME/CFS similar symptomatic in seronegative and seropositive SARS-CoV-2 children and suggest that containment measures and reduction of social contacts during 2020 and 2021 reflect on these results(6,7).

Independent observational studies conclude that the main symptoms/diagnoses of PASC among children are exertional dyspnea, cough and exercise intolerance, loss of taste/smell, fatigue and to a lesser extend muscle weakness and chest pain (8–14).

Studies in adult subjects with PASC manifestation suggest chronic stimulation of the immune system as a result of antigen persistence, autoimmunity or microbiota-gut-brain axis dysregulation(15–17). However, data regarding the pathology and immune mechanisms behind pediatric PASC is scarce. To address these knowledge gaps and the contribution of immunity, including humoral and cellular response in pediatric PASC pathogenesis, we performed a comprehensive immune profiling of 17 pediatric patients with PASC. As control we used 13 healthy COVID-19 convalescent children.

Materials and methods

Study participants

We included 17 pediatric patients with PASC (further referred as PASC) and 13 convalescent COVID-19 pediatric subjects without PASC symptomatic (further referred as control) treated in the Pediatric Infectious Diseases, Outpatient Clinic at the Department of Pediatrics I, University Hospital Essen from April 2021 to December 2021. Seventeen patients, who met the criteria of long COVID according to the German Association of the Scientific Medical Societies (AWMF) and NICE guidelines (18,19) were recruited as PASC cohort. Patients with at least two symptoms, which could not be explained by an alternative diagnosis were included. The study was approved by the Ethics Committee of the University Hospital Essen (21-9998_1-KOBO & 22-10581-BO). Written informed consent was obtained from all participants/caretakers. Demographic and clinical characteristics are provided in tables 1 and 2.

Clinical Diagnostic Workup

As previously described(20), a detailed medical history, blood tests, physical examination, oxygen saturation and blood pressure were obtained from all patients. Body mass indices (BMI; kg/m²) were assessed using BMI-centiles adapted to age, sex, and race. All further consultations with other medical specialists were conducted based on clinical presentation. Electroencephalogram (EEG), electrocardiogram (ECG), echocardiography, ultrasound of the abdomen and thyroid, chest-X-rays, lung function with lung clearance index (LCI) measurement, cranial or spinal magnetic resonance imaging, and Nerve Conduction Velocity (NCV) were performed, if necessary, based on clinical presentation. Demographic variables, preexisting diagnoses, and newly detected underlying diseases as well as long COVID-associated symptoms were documented. PASC associated symptoms were assessed through a questionnaire (see supplementary file) and/or medical

history. Shortness of breath/exercise intolerance were associated via the six-minute walking test. Patients were systematically queried as to symptoms associated with long COVID or other somatic symptoms were included in the evaluation. An improvement was defined by less reported intensity, quantity of symptoms, and/or alleviation of restrictions in daily routines, and numbers of symptoms or less restrictions in daily routines caused by long COVID symptoms.

Preparation of PBMCs

As previously described (21), peripheral blood was collected in S-Monovette K3 EDTA blood collection tubes (Sarstedt). Collected blood was prediluted in PBS/BSA (Gibco) at a 1:1 ratio and underlaid with 15 mL of Ficoll-Paque Plus (GE Healthcare). Tubes were centrifuged at 800g for 20 min at room temperature. Isolated PBMCs were washed twice with PBS/BSA and stored at -80 °C until use. The cryopreserved PBMCs were thawed by incubating cryovials 2-3 minutes at 37 °C in bead bath, washed twice in 37°C RPMI 1640 media (Life Technologies) supplemented with 1% penicillin-streptomycin-glutamine (Sigma-Aldrich), and 10% fetal calf serum (FCS) (PAN-Biotech) medium, and incubated overnight at 37 °C.

Flow cytometry - Measurement of SARS-CoV-2 reactive T cells

As previously described, PBMCs were plated in 96-U-Well plates in RPMI 1640 media (Life Technologies) (22,23). Each well was stimulated with wildtype (WT) S-protein (Miltenyi Biotec) or left untreated as a control for 16 h. The proteins were dissolved per manufacturer's directions. As a positive control, cells were stimulated with staphylococcal enterotoxin B (1 µg/mL, Sigma-Aldrich). After 2 h, brefeldin A (1 µg/mL, Sigma-Aldrich) was added. Detailed listing of the antibody panels for general phenotyping and T cell activation *ex vivo* is in Table S2. The PBMCs stimulated overnight were stained with optimal concentrations of antibodies for 10 min at room temperature in the dark. Stained cells were washed twice with PBS/BSA before preparation for intracellular staining using the Intracellular Fixation & Permeabilization Buffer Set (Thermo Fisher Scientific) as per the manufacturer's instructions. Fixed and permeabilized cells were stained for 30 min at room temperature in the dark with an optimal dilution of antibodies against the intracellular antigen. All samples were immediately acquired on a CytoFLEX flow cytometer (Beckman Coulter). Quality control was performed daily using the recommended CytoFLEX daily QC fluorospheres (Beckman Coulter). No modification to the compensation matrices was required throughout the study. Antigen-reactive responses were considered positive after the non-reactive background was subtracted, and more than 0.01% were detectable. Negative values were set to zero.

SARS-CoV-2 neutralization assay

As previously described(22), for the virus neutralization assay, sera were incubated for 30 min at 56°C in order to inactivate complement factors. Single cycle VSV*ΔG(FLuc) pseudoviruses bearing the SARS-CoV-2 WT S (D614G) protein in the envelope were incubated with quadruplicates of two-fold serial dilutions of immune sera in 96-well plates prior to infection of Vero E6 cells (1x10⁴ cells / well) in DMEM + 10% FBS (Life Technologies). At 18 hours post infection, firefly luciferase (FLuc) reporter activity was determined as previously described S5 using a CentroXS LB960 (Berthold) and the reciprocal antibody dilution causing 50% inhibition of the luciferase reporter was calculated (PVND50).

Statistics

Flow cytometry data were analyzed using FlowJo version 10.6.2 (BD Biosciences); gating strategy is presented in figures S1. For the analysis of SARS-CoV-2 reactive T cells, a threshold of 0.01% was employed to define a detectable response. Single stains and fluorescence-minus-one controls were used for gating. Gates of each study participant were adjusted according to the negative control. CD4+ T cells expressing CD154 and CD137 and CD8+ T cells expressing CD137 were defined as reactive T cells. Statistical analysis was performed using GraphPad Prism v7. Categorical variables are summarized as numbers and frequencies; quantitative variables are reported as median and interquartile range. Normality tests were performed with the Shapiro-Wilk Test. All applied statistical tests are two-sided. Kruskal-Wallis Test and Mann-Whitney-Test were applied to perform comparisons. The age between the two cohorts was compared using unpaired

two-tailed t-test, and gender was compared using two-tailed Fisher's exact test. Correlational relationships were explored with Spearman's test. p values below 0.05 were considered significant; only significant p values are reported in the figures. p values were not corrected for multiple testing, as this study was of an exploratory nature.

Results

1 .Demographic and clinical characterization of the study groups

Our study group comprised 17 convalescent COVID-19 children with PASC manifestation (further referred as PASC) and 13 convalescent COVID-19 children without PASC manifestation (further referred as control). All study participants had a negative SARS-CoV-2 nasal swab tested via RT-PCR on recruitment. During the acute phase of COVID-19 disease 100% of both study groups presented moderate/asymptomatic COVID-19 disease severity without need for hospitalization. The median COVID-19 convalescence time was 5 and 3 months for the PASC and the control study group respectively (PASC range 2-11 months, control 2-8 months) (unpaired t test PASC vs control $p=0.89$). All children participated did not receive a COVID-19 vaccine. The median age of PASC study group was 11 years (range 3-18 years) and of the control cohort 12 years (range 6-15 years) (unpaired t test PASC vs control $p=0.39$). The PASC and control cohorts comprised of 53% ($n=9$) and 15% ($n=2$) female participants, respectively and showed no significant gender difference (Fisher's exact test, $p=0.0575$). The demographic characteristics of the study cohorts are presented in Table 1.

The most common PASC symptoms among the PASC study group were shortness of breath/exercise intolerance and paresthesia with 71% ($n=12$) and 59% ($n=59$) respectively, while smell/taste disturbance, chest pain, dyspnea, headache and concentration disturbance were also quite common (29 to 41% of the PASC study cohort) (table 2). The blood count (hemoglobin, leukocytes & thrombocytes) and the clinical chemistry (AST/GOT, GPT/ALP, γ -GT, creatinine, D-dimers, CRP) excluding CK showed no statistical differences among the study groups (table 2). However, the control study group showed significantly higher CK-levels compared to PASC subjects ($p=0.011$, Mann Whitney Test) (median 153, range 113-253, normal $<170U/l$). This result may reflect more intense physical/athletic activity among the control study group due to the absence of PASC symptomatic.

2. Persistent SARS-CoV-2 reactive T cell response characterized by low avidity among the PASC subjects

In order to assess the contribution of SARS-CoV-2 S triggered persistent inflammation in PASC pathogenesis, we analyzed SARS-CoV-2 reactive T cell immunity. As the majority of the study participants were infected with either the WT or the alpha variant, we addressed the WT S reactive CD4+ and CD8+ T cell response. CD4+CD154+CD137+ and CD8+CD137+ T cells are defined as reactive CD4+ and CD8+ T cells, respectively. PASC subjects showed significantly higher frequencies of WT reactive CD4+ T cells compared to controls (Mann Whitney test $p=0.008$) (fig. 1A). WT reactive CD8+ T cell frequencies among the PASC study group were higher compared to controls, however, without statistical significance (fig. 1D).

As previously described and applied (22,24,25), we performed additionally an analysis of the functional avidity among reactive CD4+ and CD8+ T cells (gating strategy, fig. S1) using CD3low (high avidity) and CD3high (low avidity) expression as discrimination marker. We detected similar frequencies of WT reactive CD4+CD3low reactive T cells among PASC and controls (fig. 1B). However, the PASC study group presented higher frequencies of low avidity WT reactive CD4+CD3high T cells (Mann Whitney test $p=0.003$) (fig. 1C). Furthermore, we detected significantly higher frequencies of high avidity WT reactive CD8+CD3low T cells among the controls compared to PASC study group (Mann Whitney test $p=0.007$) (fig.1E), while low avidity WT reactive CD8+CD3high T cell frequencies were significantly higher among PASC subjects compared to controls (Mann Whitney test $p=0.015$) (fig.1F).

As next, we assessed the functionality of WT specific T cells regarding their cytokine production. PASC subjects showed significantly higher frequencies of WT reactive CD4 T cells producing TNF α compared to

controls (Mann Whitney test $p=0.016$) (fig. 2B). Additionally, WT reactive CD8+ T cells producing IFN γ were significantly higher among the PASC study group (Mann Whitney test $p=0.0045$) (fig. 2G). Other subsets did not show any statistical difference in the cytokine-producing T cells (Fig. 2 A, C-F, H).

3. PASC subjects´ neutralizing potential is slightly inferior compared to controls

High neutralizing antibodies titers are considered to protect effectively against SARS-CoV-2 infection, and their waning is related with high risk of reinfection or vaccine breakthrough infection(26). Currently it is unclear, whether the neutralizing antibodies (NAb) play a role in PASC pathogenesis, therefore we measured the titers of WT NAb in PASC and control individuals. We found slightly higher titers of WT NAb among the controls compared to PASC subjects, however without reaching a statistical significance (fig. 3).

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At last, we explored potential correlations between clinical diagnostic parameters and S-reactive CD4+ or CD8+ T cell frequencies among the PASC study group. We found moderate positive correlation with statistical significance between GOT, GPT and IFN γ producing reactive CD4 T cells as well as between creatinine and TNF α producing reactive CD4+ T cells (table S2). Of interest, we demonstrate a statistically significant positive correlation between CRP and IFN γ producing reactive CD8+ T cells (table S2).

Discussion

In this study, we performed an immune profiling of the cellular and humoral immune response in 17 pediatric patients with non-specific PASC. We demonstrate an increased SARS-CoV-2 CD4+ and CD8+ T cell response characterized by low avidity, TNF α and IFN γ production among PASC patients. These findings might indicate a possible role of SARS-CoV-2-specific cellular immunity that triggers inflammation as possible immune mechanism of pediatric PASC. While immunological data among pediatric PASC patients are scarce, accumulating data regarding the pathogenesis of PASC in adult cohorts indicate expansion of cytotoxic CD8+ T cells to be an important pathogenic component of PASC (15,27–30). Bacher et al have already demonstrated the relevance of low avidity S-reactive T cells in immunopathogenesis of acute SARS-CoV-2 infection(31), while our group recently demonstrated increased number of SARS-CoV-2 reactive CD8+ T cells with a low TCR avidity associated with adult PASC(32).

Although the clinical laboratory workout showed comparable creatinine and liver parameters between the studied cohorts, we found association between GOT, GPT and IFN γ producing reactive CD4 T cells as well as between creatinine and TNF α producing reactive CD4+ T cells. These results may suggest a possible role of SARS-CoV-2-reactive T cells producing inflammatory cytokines in the frame of pediatric PASC with hepatic and renal involvement. In agreement with these findings, post-COVID-19 cholangiopathy has been increasingly reported in adults(33,34), while Cooper et al described recently PASC liver manifestation in 5 pediatric patients(35).

Of interest, we demonstrated a strong positive correlation between CRP and IFN γ producing reactive CD8+ T cells. The inflammatory immune response and cytokine levels have been associated with both depression and fatigue in a large body of literature across different disorders(36–40). In the frame of adult PASC, elevated pro-inflammatory proteins in cerebrospinal fluid, microglia activation markers and persistent loss of oligodendrocytes and myelinated axons in PASC patients and mice with neurological PASC manifestation have been demonstrated (41,42), while Kiho et al showed TNF- α and C-reactive protein predicted strong association with PASC symptoms at 12 months (43).

The present study has limitations. The number of included patients was small so that we were unable to differentiate immune response according to symptom clusters. Rao et al in a large-scale exploratory study found that the burden of pediatric PASC on the health system was low(10). However, despite its phenomenal benignity in most of the cases, long-term psychological and somatic effects of pediatric PASC are currently unknown. Our study group underlined the risk for the development of restrictive eating disorders in children and adolescents with long-COVID-associated smell and taste dysfunction(20). Pediatric PASC is a public health and social issue requiring interdisciplinary vigilant attention.

Taken together, our results suggest that cellular inflammatory response triggered by SARS-CoV-2 may be responsible for the observed sequelae in pediatric PASC. These results may have implications on future therapeutic and prevention strategies.

Study Approval

The study was approved by the Ethics Committee of University Hospital Essen (21-9998.1-KOBO, 20-9791-BO & 22-10581-BO). Written informed consent was obtained from all participants.

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Tables

Table 1: Demographic characteristics of the study cohorts

	PASC (N=17)	Control (N=13)	p value
Age years -median (range)	11 (3-18)	12 (6-15)	0.39
Female gender N (%)	9 (53)	2 (15)	0.0575
Time since COVID-19 Diagnosis (months)	5 (2-11)	3 (2-8)	0.89
Duration of PASC symptoms (months)*	5 (2-11)	N/A	N/A
COVID-19 Severity N (%)	COVID-19 Severity N (%)	COVID-19 Severity N (%)	
<i>Moderate</i>	17(100)	13(100)	N/A
SARS-CoV-2 vaccination	0	0	N/A
Variant of concern N (%)	Variant of concern N (%)	Variant of concern N (%)	
<i>WT</i>	10 (59)	7(54)	1
<i>Alpha</i>	7 (41)	6 (46)	0.8167
PASC Type	PASC Type	PASC Type	
Ongoing COVID-19	0	N/A	N/A
Post-acute COVID-19 Syndrome	17 (100)	N/A	N/A

*Months from SARS-CoV-2 Diagnosis (positive PCR) till the time point of study recruitment

Table 2: Clinical characteristics of the study cohorts

	PASC (N=17) median (range)	Controls (N=13) median (range)	p value	
Blood parameters	Blood parameters	Blood parameters	Blood parameters	Blood parameters

	PASC (N=17) median (range)	Controls (N=13) median (range)	p value	
BMI (kg/m ²)	18 (14.3-23.7)	19 (13-26)	p=0.669	
Leukocytes/nl	6.44 (4.13-11.42)	5.73 (4.73-9.29)	p=0.426	
Hb (g/dl)	13 (11-14.5)	13.4 (11-15.4)	p=0.836	
Thrombocytes/nl	291 (189-465)	270.5 (226-351)	p=0.967	
Creatinine (μmol/l)	55 (18-78)	48 (35-72)	p=0.095	
AST/GOT (U/l)	23 (12-34)	27 (17-36)	p=0.094	
ALT/GPT (U/l)	18 (9-30)	19 (9-36)	p=0.503	
γ-GT (U/l)	13 (7-24)	14 (12-19)	p=0.205	
Creatine Kinase (U/l)	101 (15-230)	153 (113-253)	p=0.011	
Ddimers (mg/l)	0.22 (0.19-0.54)	0.2 (0.2-0.6)	p=0.802	
CRP (mg/dl)	0.4 (0.02-0.5)	0.1 (0.0-0.5)	p=0.342	
PASC Symptoms N(%)	PASC Symptoms N(%)	PASC Symptoms N(%)	PASC Symptoms N(%)	PASC Symptoms N(%)
Shortness of breath/exercise intolerance	12 (71)	N/A	N/A	
Paresthesia	10 (59)	N/A	N/A	
Smell/taste disturbance	7 (41)	N/A	N/A	
Chest pain	6 (35)	N/A	N/A	
Dyspnea	5 (29)	N/A	N/A	
Headache	5 (29)	N/A	N/A	
Concentration disturbance (brain fog)	5 (29)	N/A	N/A	
Vertigo	4 (24)	N/A	N/A	
Fatigue	2 (12)	N/A	N/A	
Diarrhea	2 (12)	N/A	N/A	
Sleep disturbances	1 (6)	N/A	N/A	
Sensory disorder	1 (6)	N/A	N/A	
Palpitations	1 (6)	N/A	N/A	
Abdominal pain	1 (6)	N/A	N/A	
New diagnosis since COVID-19	New diagnosis since COVID-19	New diagnosis since COVID-19	New diagnosis since COVID-19	New diagnosis since COVID-19
Eating disorder	1 (6)	N/A	N/A	
Depressive disorder	1 (6)	N/A	N/A	
Asthma exacerbation	1 (6)	N/A	N/A	
Increased susceptibility to infections	1 (6)	N/A	N/A	
Instrumental Diagnostics N (%)	Instrumental Diagnostics N (%)	Instrumental Diagnostics N (%)	Instrumental Diagnostics N (%)	Instrumental Diagnostics N (%)
EKG <i>normal</i>	11 (65)	0	N/A	
	11 (65)	N/A	N/A	

	PASC (N=17) median (range)	Controls (N=13) median (range)	p value	
NLG	11 (65)	0	N/A	
<i>normal</i>	10 (65)	N/A	N/A	
<i>pathological</i>	1 (6)	N/A	N/A	
Echocardiogram	11 (65)	11 (85)	N/A	
<i>EF normal</i>	11 (65)	11 (85)	N/A	
<i>mild TI</i>	8 (47)	3 (27)	0.0861	
<i>mild AI</i>	1 (6)	0	1	
<i>mild MI</i>	0	2 (15)	0.476	
<i>Not relevant</i>	0	1 (8)	1	
<i>pericardial</i>				
<i>effusion</i>				
Lung function test	9 (53)	0	N/A	
<i>Ventilatory disorder</i>	1 (6)	N/A	N/A	
<i>Bronchial hyperreactivity</i>	1 (6)	N/A	N/A	
<i>Hyperinflation</i>	1 (6)	N/A	N/A	
Comorbidities	Comorbidities	Comorbidities	Comorbidities	Comorbidities
Asthma bronchiale	1 (6)	0	N/A	

Figure legends

Figure legends

Figure 1: SARS-CoV-2 CD4+ driven T cell response characterized by low avidity among the PASC subjects. Characterization of SARS-CoV-2 S-reactive T cells in PASC and control subjects. Blood samples of 17 pediatric PASC patients and 13 convalescent controls were stimulated with SARS-CoV-2 S-WT and analyzed by flow cytometry. (A) Frequencies of WT-reactive CD4+ T cells among PASC and controls. (D) Frequencies of WT-reactive CD8+ T cells among PASC and controls. (B & E) Frequencies of WT reactive CD4+CD3low+ and CD8+CD3low+ T cells. A high functional avidity of SARS-CoV-2 reactive T cells was approached by determining the CD3low+ cells among CD4+CD154+CD137+ and CD8+CD137+ cells. (C & F) Frequencies of WT reactive CD4+CD3high+ and CD8+CD3high+ T cells. A low functional avidity capacity of SAR-CoV-2 reactive T cells was determined by detecting CD3high+ cells among CD4+CD154+CD137+ and CD8+CD137+ cells. Unpaired data were compared with Mann-Whitney-test. P<0.05 was considered significant, only significant p values are documented in the figures.

Figure 2: Analysis of cytokine-producing SARS-CoV-2 reactive T cells. The frequencies of IL2, IFN γ , TNF α or GrB producing WT-reactive T cells were analyzed among PASC subjects and controls. (A & E) IL2 producing SARS-CoV-2 reactive CD4+ and CD8+ T cells. (B & F) TNF α producing SARS-CoV-2 reactive CD4+ and CD8+ T cells. (C & G) IFN γ producing SARS-CoV-2 reactive CD4+ and CD8+ T cells. (D & H) GrB producing SARS-CoV-2 reactive CD4+ and CD8+ T cells.

Figure 3: Analysis of neutralizing capacity of humoral immunity in pediatric PASC compared to controls. Analysis of WT S NAb titers of both study groups. Scatterplots show line at median. Unpaired data were compared with Mann-Whitney-test. P<0.05 was considered significant, only significant p values are documented in the figures.



