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Evidence of lung perfusion defects and ongoing inflammation in an adolescent with post-acute sequelae of SARS-CoV-2 infection

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Although more patients are surviving COVID-19, there are emerging data on a substantial proportion of patients with persisting, and often debilitating, symptoms and sequelae for months following acute COVID-19. This scenario is commonly referred to as post-acute sequelae of SARS-CoV-2 infection (PASC) or long COVID.[1] Some of the most commonly reported symptoms are fatigue, weakness, breathlessness, chest pain, and concentration impairment. Several independent studies have shown the existence of PASC, with chronic inflammation and chronic endothelial disease suggested among the possible pathophysiological mechanisms of this multifaceted condition.[1] Although the course of acute SARS-CoV-2 infection in children is normally milder and can present with less typical symptoms compared with adults,[2,3] emerging evidence from Sweden,[4] Italy,[5] the UK,[6] and Russia[7] suggests that children can also have persisting symptoms more than 3 months after acute COVID-19, with a similar pattern of symptoms as reported by adults. In light of an increasing number of parents and clinicians asking for guidance and support for children with PASC in our centre, we have set up a post-COVID Paediatric Unit. Children with a microbiologically confirmed diagnosis of COVID-19 are assessed in our post-COVID Paediatric Unit at least 5 weeks after the onset of acute infection (see the appendix for our post-COVID paediatric follow-up protocol).

Here we present the case of a 14-year-old girl initially diagnosed with mild COVID-19 in October, 2020, during the midst of the second peak in Rome, Italy. During the acute phase, she presented with a 1-day history of low-grade fever (37·3°C), rhinitis, anosmia, and ageusia. Before the infection, she was a playful, active girl with no comorbidities, who loved to ride horses and play music. She had never previously had any cardiorespiratory problems (eg, asthma, tachycardia, chest pain), neither at rest nor under physical or emotional stress. After the acute COVID-19 phase, around 30 days post-onset, she developed persistent headache, chest pain, fatigue, and tachycardia. Due to the feeling of tachycardia with chest pain, she presented to the paediatric emergency department multiple times. She underwent clinical investigations, including serial electrocardiograms (ECG) and biochemical assessments (eg, markers of myocardial injury, coagulation profile, and inflammatory markers), which were all negative. Thyroid function was normal, and there were no signs of other ongoing or recent bacterial, viral, or parasitic infections detected. The family paediatrician did an echocardiogram, which was normal. Holter ECG and stress ECG showed only sinus tachycardia and poor tolerance to physical exertion, with no tachyarrhythmias. Bronchopneumological evaluation was also performed: both baseline and post-bronchodilator spirometry showed normal lung flows and volumes. No abnormalities were found on a chest CT scan performed without contrast.

Due to the persistence of symptoms 7 months after the onset of COVID-19, she was admitted in May, 2021, to Fondazione Policlinico Universitario A Gemelli IRCCS, a large university hospital in Rome, Italy, recognised as one of the COVID-19 referral hospitals in the region. During the admission she underwent further assessment, including a 6-min walk test that showed tachycardia within 1 min (up to 155 beats per min) and easy fatigability with the need to rest four times during the test. Holter ECG was normal with the exception of tachycardia. We did a cardiac MRI scan, which was normal. Stress spirometry showed poor exercise tolerance, but with normal lung flows and volumes. Daytime and sleep oximetry monitoring using polysomnographic monitoring showed normal saturation values, with no signs of obstructive or central apnoeas during sleep. A psychological assessment using the Wechsler Intelligence Scale for Children (fourth edition), and a personality and global operation questionnaire (the Child Behavior Checklist for Ages 6–18 by Thomas M Achenbach), found a supportive family context, higher than average IQ, and normal psychological and cognitive test results.

Routine blood tests were normal; however, additional immunological assessments revealed high concentrations of IL-6 (633 pg/mL), IL-1 (8·55 pg/mL), and TNFα (32·7 pg/mL), as well as unusual B-cell and regulatory T-cell patterns, shifted towards a high ratio (22·7) of effector T cells to regulatory T cells, and low concentrations of $CD27^+$ memory B lymphocytes, suggesting a pro-inflammatory background (figure 1). 7 months after the diagnosis of SARS-CoV-2 infection, our patient also had high concentrations of IgA and IgG anti-SARS-CoV-2 antibodies.

Due to the persisting, objective tachycardia and fatigue, the absence of a psychological cause, and evidence of a proinflammatory immune profile in our patient, together with emerging evidence of potential vascular damage in adults, we decided to perform cardiopulmonary exercise testing (CPET): this is a dynamic, non-invasive assessment

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See Online for appendix

of the cardiopulmonary system at rest and during exercise. The CPET was done on a cycle ergometer with a 7 watt per min ramp protocol. The Hansen–Wasserman equation was used to calculate the predicted normal values. The test showed a slight to moderate reduction in functional capacity (peak VO_2 was 24·4 mL/kg/min, which corresponds to 68% of predicted VO_2 max) secondary to reduced cardiovascular efficiency, with initial indirect signs of increased pulmonary vascular resistance (VE/VCO_2 slope of 36) with reduced partial pressure of end-tidal CO_2, suggestive of pulmonary hypertension. The test confirmed the presence of sinus tachycardia (maximum heart rate 180 beats per min) and the absence of ECG changes, symptoms of reduced coronary flow reserve, or significant arrhythmias. In addition, the test showed no signs of ventilatory limitation (breathing reserve 56·7%), with oxyhaemoglobin saturation at peak effort equal to 99%.

A recent Viewpoint from Dhawan and colleagues proposed a novel approach for assessing patients with long-term COVID-19 sequelae.[8] The authors hypothesised that some patients with PASC might have pulmonary vascular sequelae with residual clot burden, small vessel injury, and subsequent haemodynamic sequelae potentially explaining at least some of the persisting symptoms. They suggested that ventilation–perfusion single photon emission computed tomography (V/Q SPECT), the gold-standard screening test in the assessment of chronic thromboembolism, might be useful for assessment of chronic lung perfusion defects in patients with PASC. SPECT is a well established imaging tool that can process and reconstruct the signal (gamma rays detected by a gamma camera) in three-dimensional images, thereby providing improved detection and a more accurate localisation of dysfunctional findings compared with planar images. Following this recent evidence and considering the clinical picture of our patient, we referred the patient to our Nuclear Medicine Unit for planar lung scintigraphy with ^{99m}Tc-macroaggregated albumin as the perfusion tracer, followed by SPECT with co-registered CT (SPECT/CT) acquisition. The dose activity (141 MBq) was prepared just before the injection according to the latest guidelines from the European Association of Nuclear Medicine for lung scintigraphy in children.[9] The patient was intravenously injected in the supine position immediately before starting the planar scan, which consisted of anterior, posterior, lateral right, lateral left, oblique posterior right, and oblique posterior left projections, followed by lung SPECT/CT acquisition. Whereas planar scintigraphy did not show areas with decreased radiopharmaceutical uptake in both lungs, a significant perfusion defect was observed on SPECT images in the apical segment of the right upper lobe, which did not correspond to parenchymal alterations on co-registered CT slices (figure 2). These findings could suggest microvascular level involvement, most likely associated with endothelial damage, as hypothesised in other studies in adults.[8]

CPET detected initial signs of pulmonary hypertension with minimal overload of the right ventricle, which might

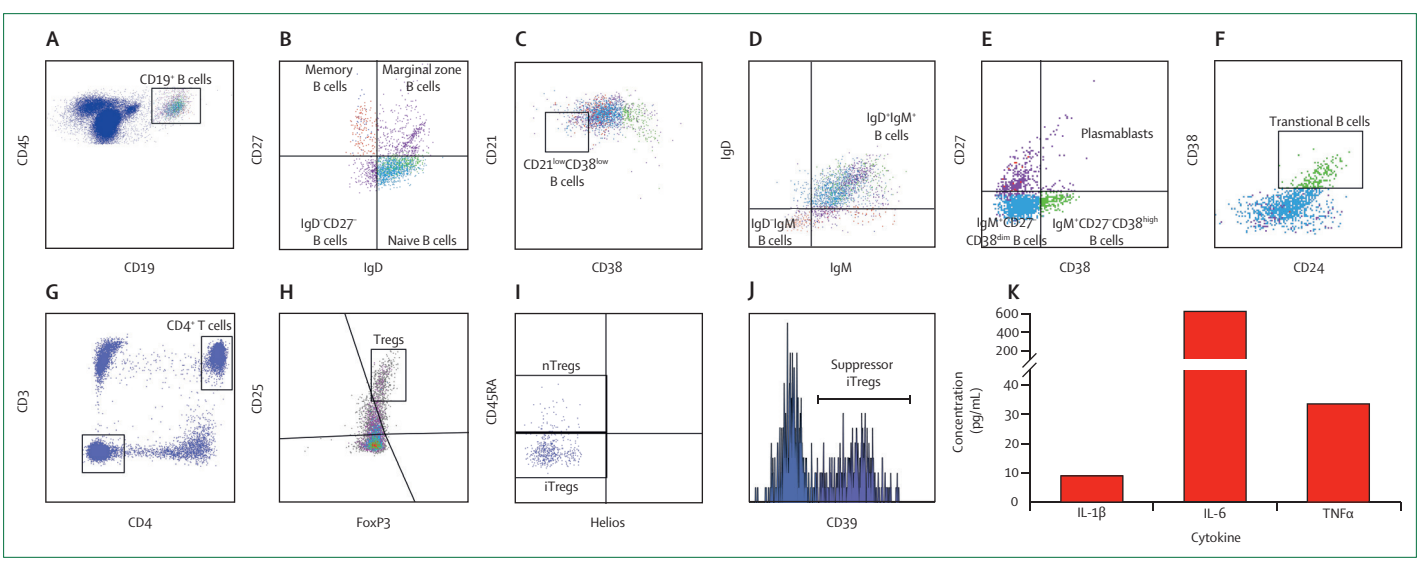

Figure 1: **B-cell and regulatory T-cell subpopulations and inflammatory cytokine concentrations**
B-cell and T-cell panels were analysed through a 13-colour Cytoflex, by use of, respectively, the 8-colour DuraClone IM B cells Tube and IM Treg Tube (Beckman Coulter). B-cell panel (A–F): after gating lymphocytes according to forward *vs* side scatter (not shown), B cells were gated by CD19 *vs* CD45 staining (A); by staining for CD27 and IgD, naive $IgD^+IgM^+CD27^-$ B cells, $IgD^+IgM^+CD27^+$ marginal zone B cells, and $IgD^-IgM^-CD27^+$ switched memory B cells can be distinguished (B). The staining for CD21 and CD38 expression allows the additional distinction of $CD38^{low}CD21^{low}$ B cells (C); by staining for IgM and IgD (D) it is possible to discriminate between $CD38^{+++}IgM^-$ plasmablasts (E) and $CD38^{++}IgM^{high}$ transitional B cells (F); and by staining for CD138 and for CD24, plasma cells and regulatory B cells (not shown) are, respectively, distinguishable. Regulatory T-cell panel (G–J): $CD4^+$ T cells were gated by CD3, CD4, CD8, and CD45 staining (G), and further detailed with $CD45RA^-$ staining; after gating $CD127^{low}$ and $CD25^{high}$ T helper cells, it was possible to distinguish $FoxP3^+$ regulatory T cells (H), and among them discriminate between natural (I), inducible (I), and suppressor (J) regulatory T cells, staining with CD39 and Helios. (K) Inflammatory cytokine concentrations were measured in serum samples taken from the patient on admission, by use of the ELLA Assay (Bio-Techne, from R&D Systems). Treg=regulatory T cell. nTreg=natural regulatory T cell. iTreg=induced regulatory T cell.

explain the compensatory tachycardia experienced during submaximal effort. Both lung SPECT/CT and CPET provided results suggestive of a pulmonary circulation dysfunction with possible peripheral microvascular and endothelial damage. Furthermore, results from CT and respiratory function tests ruled out a ventilation-associated impairment.

To our knowledge, this is the first reported evidence of organ dysfunction in an adolescent with PASC. The evidence of this dysfunction was unexpected and, after a comprehensive and multidisciplinary assessment that actively involved the patient and both parents in the clinical decision-making process, the decision was made to treat the girl with oral steroids (methylprednisolone 2 g/kg once daily for 2 weeks, then tapered over the next 4 weeks, along with a proton pump inhibitor) and low molecular weight heparin (enoxaparin 6000 units twice daily, with a plan of changing to oral anticoagulants and continuation of therapy for 6–9 months in total). The decision was based on the presence of inflammation and lung perfusion defects consistent with microemboli caused by microvascular and endothelial damage, which we hypothesised might be caused by a chronic inflammatory stimulus. We made a plan to follow up with a lung SPECT/CT scan and CPET 3–6 months after the beginning of the treatment, along with a comprehensive clinical assessment and full pulmonary and cardiovascular functional evaluation.

Currently, there is no definite evidence on the causes of or underlying risk factors for PASC. The B-cell pattern in our patient is similar to that identified in a small case-control study (preprint) that compared 12 children with PASC with 17 children who had fully recovered from COVID-19.[10] This immunological pattern, characterised by consistent amounts of plasmablasts and proinflammatory cytokines, might explain why some children develop PASC. Dysfunction or inflammation of the endothelium and autoimmune events triggering chronic inflammation have been suggested.[1] A further study found that patients with COVID-19 have pronounced increases in autoantibody reactivities compared with uninfected controls, with a high prevalence of autoantibodies against immunomodulatory proteins including cytokines, chemokines, complement components, and cell surface proteins.[11] The authors also established that these autoantibodies perturb immune function and impair virological control by inhibiting immunoreceptor signalling and by altering peripheral immune cell composition. We hypothesise that similar events might explain the persisting symptoms and the objective findings in our patient.

Assessing PASC in children and adolescents is especially challenging and requires a close partnership between paediatricians, specialists with experience managing COVID-19 and PASC (including cardiologists, haematologists, pneumologists, rheumatologists, radiologists, and immunologists), and international experts. Patients and their families play a pivotal role in recognition of the sequelae, and in clinical diagnostic and care decision making; this is especially important in light of the limited evidence base and lack of guidelines. Our protocol is designed in a step-by-step approach to assess and triage children for further investigations and specialist support on the basis of presenting symptomatology, thereby reserving the most complex and invasive tests for the most severe cases, reducing unnecessary exposure, and optimising benefit to risks (appendix).

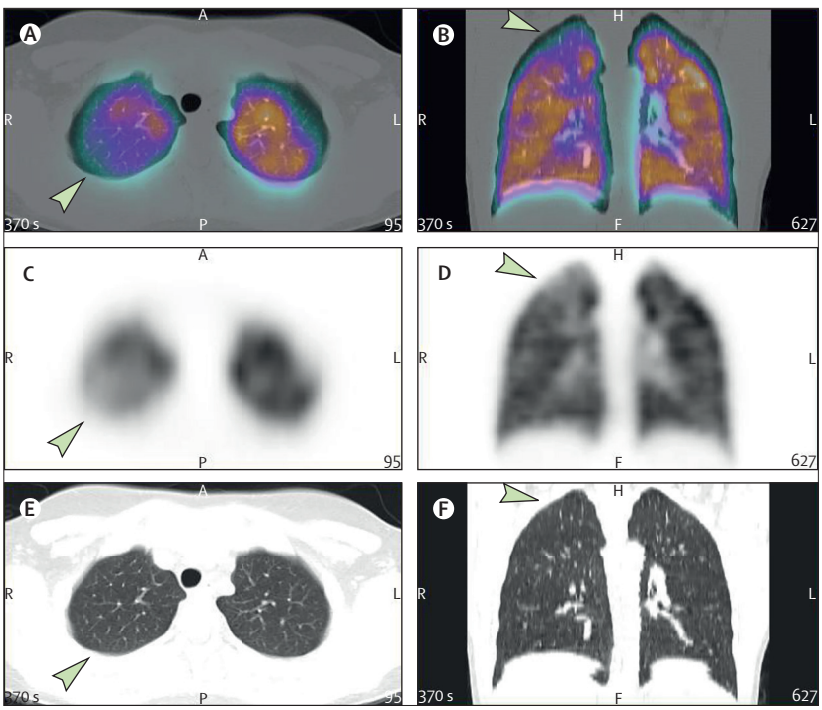

Figure 2: **Lung SPECT/CT**
Lung SPECT/CT scan with ^{99m}Tc-macroaggregated albumin showed hypoperfusion in the apical segment of the right upper lobe, clearly evident on axial and coronal hybrid images (A, B; arrow) as well as on functional slices (C, D; arrow). This finding did not correspond to parenchymal alterations on co-registered CT images (E, F; arrow). SPECT/CT=single photon emission computed tomography with co-registered CT.

In conclusion, in this report we present the first detailed evaluation of PASC in an adolescent, providing evidence of pulmonary circulation dysfunction with possible lung microvascular or endothelial damage as detected by CPET and chest SPECT/CT. Currently, most attention has been focused on long COVID in adults, with few studies including children, resulting in a lack of case definitions and management guidelines for this population. This report supports the emerging evidence on long COVID in children and highlights that, although children generally present with mild, acute COVID-19, they are at risk of prolonged organ damage, similar to what has been identified in adults. This report highlights the importance of doing a robust assessment of children presenting with PASC. SPECT/CT is useful to detect microvascular damage in patients with more severe and persisting symptoms. We urge clinicians and policy makers to not underestimate the risk of and consequences of long-term

COVID-19 sequelae in children. We also call for urgent studies aiming for a better understanding of the PASC burden, with a particular focus on investigation of chronic organ damage to inform treatment and improve long-term COVID-19 outcomes in children. These data are urgently needed to identify risk factors for targeted prevention and support, inform management guidelines, and also provide indirect benefits to the understanding of other post-infectious conditions.

Contributors

DB and PV conceptualised and prepared the Case Report. CDR, DM, and LS contributed to the literature search, data collection, and data interpretation. DDG and DAP were responsible for the SPECT studies, from performance to interpretation, and provided crucial input to both the clinical management and the Case Report. GDS was responsible for the immunological studies. MS was responsible for the microbiological investigations. IL was responsible for the coagulation studies and for the management and follow-up of the anticoagulant treatment. FB was responsible for the CPET and its interpretation. DPRC was responsible for the psychological and psychiatric assessment of the patient. DB and PV had full access to all the data and verified it, and had final responsibility for the decision to submit for publication. PV, DB, DM, and LS supervised the manuscript preparation and critically reviewed and edited the final manuscript.

Declaration of interests

We declare no competing interests.

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