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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Buonsenso D, Giuda D, Sigfrid L, et al. Evidence of lung perfusion defects and ongoing inflammation in an adolescent with post-acute sequelae of SARS-CoV-2 infection. *Lancet Child Adolesc Health* 2021; published online July 30. https://doi.org/10.1016/S2352-4642(21)00196-6.

Current post-COVID paediatric follow-up

Children with a microbiologically confirmed diagnosis of COVID-19 are assessed in our post-COVID Paediatric Unit at least five weeks after the onset of acute infection (see supplementary figure below). They are screened using the International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) standardised, open access COVID-19 paediatric follow-up survey to assess for the presence of persistent symptomatology or sequelae, to inform referrals for further clinical investigations and support. Although this survey was initially developed for research purposes, it also turned out to be a very useful tool for clinical assessment of persistence of symptoms in children with previous SARS-CoV-2 infection, being a valid model of integration of research into practice. Data are also collected on an electronic database for an observational longitudinal follow-up study assessing long-term COVID-19 outcomes in adults and children, integrating research into clinical practice.

Patients assessed in the post-COVID Paediatric Unit

The patient population consists of children diagnosed with COVID-19 and admitted to our hospital, or diagnosed in our paediatric emergency department and discharged, and/or assessed by a general practitioner and referred to the post-COVID Paediatric Unit. These patients enter the step 1 assessment and then enter the next steps according to the results of the previous step assessment.

Inclusion criteria:

- Children aged 0-18 years
- The child sought/needed primary or secondary medical care for COVID-19
- Laboratory (RT-PCR) diagnosis of COVID-19
- At least 28 days from the onset of COVID-19 symptoms
- Parent's/carer's/guardian's consent to participate

Step 1 assessment

This phase is characterised by a routine clinical examination with the main vital parameters and the administration of the paediatric long COVID survey developed by ISARIC (https://isaric.org/research/covid-19-clinical-research-resources/paediatric-follow-up/); the survey is administered to all children aged less than 18 years with a microbiologically confirmed diagnosis of SARS-CoV-2 infection with a PCR test on nasopharyngeal swab, and is administered in the outpatient setting by a paediatrician.

From this cohort, those with persisting symptoms are reassessed at 3 months from initial infection and undergo a new clinical assessment and investigation of persisting symptoms with the ISARIC paediatric long COVID survey. Those with any of the following are included in the further steps of the follow-up:

- Low grade fever persisting for more than two weeks, without alternative causes (>37.5°C)
- Moderate/severe fatigue
- Cardiovascular symptoms (eg palpitations, syncope)
- Neurological symptoms (eg. chronic headache)
- Respiratory Symptoms (chest pain, cough, dyspnoea)

- Muscle/joint pain
- Chronic diarrhoea or abdominal pain

Step 2 assessment

Eligible children from step 1 are assessed in step 2. Parents of eligible children are invited to have the following examinations, in order to understand if the described symptoms have any accompanying organic abnormalities:

- **Routine blood tests:** complete blood count, C-reactive protein, ferritin, Vitamin D, Troponin, proBNP
- **Coagulation assessment:** coagulation profile including D-Dimer and fibrinogen
- **Immunological tests:**
    - ***Anti SARS-CoV-2 antibodies*** *(including neutralising antibodies if available)*
    - ***Cytokines analyses*** (IL-6, IL-1β, TNFα, IFNγ, IL-10, Il12p70, IL-2, IL-4), ***Flow cytometry analysis*** (CD45-KRO, CD4-PC7, CD3-APC750, CD25-PE, FoxP3-A647, Helios-PBE, CD39-PC5.5, CD45RA-FITC, CD45-KrOrange, CD19- ECD, CD27-PC7, IgD-FITC, CD38-APC750, CD21-PE, IgM-PB), MicroRNA signatures and PBMC single-cell RNA sequencing analysis to assess potential biomarkers of PASC *(within clinical studies and after approval)*
- **Stool examination:** chemical examination of stool, faecal calprotectin and microbiota assessment (if chronic diarrhoea)
- **Lung ultrasound** extended on all thoracic areas, and **heart ultrasound**
- **Global Spirometry, at rest and after stress**. On the basis of the values obtained from spirometry, the clinical and anamnestic history reported by the patient, the child may have a **bronchodilator reversibility test**: the child will have to inhale a bronchodilator drug administered by means of a spacer
- **Six-minute walk test**
- **Measurement of haemoglobin saturation:** measurement of oxygen saturation by pulse oximetry in basal conditions at rest, during and after maximal physical exercise (performed for exercise spirometry) and during and after sub-maximal physical exercise (performed for the six-minute walk test)
- **Overnight oximetry, polysomnography and capnography:** performed by transcutaneous CO2 monitoring with the Capnograph Sentec portable device. The parameters measured and taken into consideration are: monitoring time (h min); minimum tcPCO2 (mmHg); maximum tcPCO2; (mmHg) average tcPCO2 (mmHg); tcPCO2> 50 mmHg (% of time). In the absence of instrumentation for transcutaneous capnography, arterial and/or capillary EAB is performed upon awakening

- **Baseline and stress ECG**
- **Cardiopulmonary exercising testing (CPET)** with 7 Watt/min ramp protocol on cycle ergometer, using the Hansen/Wassermann equation to calculate the final normal expected values
- **Awake and sleeping Electroencephalography**, if neurological symptoms
- **Consult rheumatologist and a dermatologist** if chronic muscle-articular pain or skin lesions

Step 3 assessment

Children with abnormal findings in the step 2 assessment are enrolled in the step 3 assessment. Parents of eligible children are invited to have further examinations on a personalised basis according to the specific abnormalities:

- **Heart MRI**, if abnormal ECG on rest or under stress, or abnormal heart ultrasound
- **Chest CT scan and Lung SPECT**, if baseline or stress desaturations, or abnormal spirometry at baseline or under stress. The SPECT will be only offered in children older than nine years of age, since patient compliance is necessary for the appropriate methodology
- **Brain Magnetic Resonance Imaging**, if central apnoea at polysomnography or abnormal EEG, or persistent headache > 12 weeks. Consider brain SPECT if available and persisting neurological symptoms
- **Endoscopy**, if chronic diarrhoea > 4 weeks and signs of bowel inflammation at stool examination
- **Skin biopsy**, if chronic skin lesions

Supplementary figure