Clinical features of COVID-19 in Pediatric Patients, long term effects

(COPP2-study)

(May 2021)

PROTOCOL TITLE: ***'clinical features of COVID-19 in pediatric patients, long term effects'***.

Protocol ID	COPP-2
Short title	COVID-19 in pediatrics, follow-up.
EudraCT number	N/A
Version	10
Date	06-05-2021
Coordinator investigator/project leaders	Drs. C.L.H. Kosterink-Brackel Amsterdam University Medical Centers. Department of pediatric pulmonology, Emma Children's hospital Meibergdreef 9, H7-217, 1105 AZ Amsterdam c.l.brackel@amsterdamumc.nl +31 20 5669111 / +31 6 18745425 Dr. S. Hashimoto Amsterdam University Medical Centers, UvA. Dept. of respiratory disease & Dept. of pediatric pulmonology, Emma Children's hospital. Meibergdreef 9, F5-260, 1105 AZ Amsterdam S.hashimoto@amsterdamumc.nl +31 20 5667924 Drs. L.C.E Noij Dept. of respiratory disease & Dept. of pediatric pulmonology, Emma Children's hospital. Meibergdreef 9, F5-260, 1105 AZ Amsterdam l.noij@amsterdamumc.nl +31 6 22315290 Dr. E.G. Haarman Amsterdam University Medical Centers. Dept. of pediatric pulmonology, Emma Children's hospital Meibergdreef 9, H8-252, 1105 AZ Amsterdam eg.haarman@amsterdamumc.nl + 31 20 5669111 Drs. J. van Schuppen Amsterdam University Medical Centers. Dept of Radiology and nuclear medicine Meibergdreef 9, 1105 AZ Amsterdam j.vanschuppen@amsterdamumc.nl +31 20 5669111 Dr. L. Haverman Amsterdam University Medical Centers. Dept. of pediatric psychology Meibergdreef 9, G8-154, 1105 AZ Amsterdam l.haverman@amsterdamumc.nl +31 20 5669111 Dr. C. Longo Amsterdam University Medical Centers.

	Dept. of respiratory medicine and Dept. of Pediatric Respiratory Medicine. Meibergdreef 9, 1105 AZ Amsterdam c.longo@amsterdamumc.nl +31 20 5669111
Principal investigator(s) (In Dutch: hoofdonderzoeker)	Dr. S.W.J. Terheggen-Lagro Department of pediatric pulmonology, Emma Children's hospital, Amsterdam University Medical Centers. Meibergdreef 9, H8-256, 1105 AZ Amsterdam s.w.terheggenlagro@amsterdamumc.nl +31 20 5669111 Drs. M. Bannier Maastricht University Medical Center. Dept. of pediatric pulmonology P. Debyelaan 25, 6229HX Maastricht. +31 6 41826123 Dr. Mariëlle Pijnenburg, Assoc. Prof. Pediatric Pulmonology Erasmus MC - Sophia Children's Hospital. Dept. of Pediatric Pulmonology and Allergology Wytemaweg 90, 3015 GJ Rotterdam Room sp 3456 +31 10 7036263 m.pijnenburg@erasmusmc.nl Dr. E.P. Buddingh Department of Pediatrics, Willem-Alexander Kinderziekenhuis Leiden University Medical Centre e.p.buddingh@lumc.nl +31-71-5296658 / +31-71-5262824

	Dr. C. Longo Amsterdam University Medical Centers. Dept. of respiratory medicine and Dept. of Pediatric Respiratory Medicine. Meibergdreef 9, 1105 AZ Amsterdam c.longo@amsterdamumc.nl +31 20 5669111
Principal investigator(s) (In Dutch: hoofdonderzoeker)	Dr. S.W.J. Terheggen-Lagro Department of pediatric pulmonology, Emma Children's hospital, Amsterdam University Medical Centers. Meibergdreef 9, H8-256, 1105 AZ Amsterdam s.w.terheggenlagro@amsterdamumc.nl +31 20 5669111 Drs. M. Bannier Maastricht University Medical Center. Dept. of pediatric pulmonology P. Debyelaan 25, 6229HX Maastricht. +31 6 41826123 Dr. Mariëlle Pijnenburg, Assoc. Prof. Pediatric Pulmonology Erasmus MC - Sophia Children's Hospital. Dept. of Pediatric Pulmonology and Allergology Wytemaweg 90, 3015 GJ Rotterdam Room sp 3456 +31 10 7036263 m.pijnenburg@erasmusmc.nl Dr. E.P. Buddingh Department of Pediatrics, Willem-Alexander Kinderziekenhuis Leiden University Medical Centre e.p.buddingh@lumc.nl +31-71-5296658 / +31-71-5262824
Sponsor:	Amsterdam University Medical Centers
Subsiding party	Christine Bader Stichting Irene Kinderziekenhuis, Stichting Astma bestrijding, Stichting Steun Emma, crowdfunding, others still in review.
Independent expert(s)	Dr. J. Altenburg Department of pulmonology, Amsterdam University Medical Centers. j.altenburg@amsterdamumc.nl +31 20 5665254
Laboratory sites	Amsterdam University Medical Centers: Breathomics Prof. Dr. A.H. Maitland van der Zee Amsterdam University Medical Centers Dept. of respiratory medicine and Department of Pediatric Pulmonology, Emma Children's Hospital.

	Radiology Prof. Dr. J. Stoker Amsterdam University Medical Centers. Dept. of Radiology and nuclear medicine Respiratory physiology Prof. Dr. J.G. van den Aardweg Amsterdam University Medical Centers, Dept. of respiratory medicine and Dept. of pulmonary physiology

Protocol signature sheet:

Name	Signature	Date
Sponsor or legal representative: Prof. Dr. J.B. van Goudoever Head of the department of Pediatrics, Emma Children's Hospital, Amsterdam University Medical Centers, Amsterdam.		06-05-2021
Principal investigator: Dr. S.W.J. Terheggen-Lagro Head of the department of pediatric pulmonology, Emma Children's Hospital, Amsterdam University Medical Centers, Amsterdam.		06-05-2021

Inhoud

1.

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
BSA	body surface area
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie mensgebonden onderzoek.
Chest CT	Chest Computed Tomography (CT)
COPP	Clinical Features of COVID in Pediatric Patients (COPP Study).
COVID-19	Coronavirus Disease 2019
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
DMP	Data management plan
EKG	Electrocardiogram
GC-MS	Gas chromatography–mass spectrometry
GDPR	General Data Protection Regulation; in Dutch: Algemene verordening Gegevensbescherming (AVG)
IC	Informed Consent
IMPD	Investigation Medical Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MIS-C	Multisystem inflammatory syndrome, temporally related to SARS-CoV-2 infection.
PIMS-TS	Pediatric inflammatory multisystem syndrome, temporally associated with SARS-CoV-2.
PTS	post thrombotic syndrome
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiëne
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction.
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus type 2
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission is not regarded as the sponsor, but referred to as a subsidizing party.
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; In Dutch: Uitvoeringswet AVG.

VTE	Venous tromboembolism
WMO:	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch wetenschappelijk onderzoek met mensen.

SUMMARY

Rationale: The pandemic novel coronavirus (SARS-COV-2) causes the disease COVID-19, ranging from mild flu like symptoms to severe and potentially fatal acute respiratory distress and cardiogenic shock syndrome. In adults who recovered from COVID-19, long-term pulmonary sequela are frequently reported, causing mild to severe long term morbidity. In children, the first cohort studies show long term sequelae in 12-42% of patients. A Dutch survey among pediatricians showed 89 children with long term complaints, resulting in severe limitations in daily life in 36%.**Objective:** We aim to describe long term morbidity, and immune response at 4 to 12 months following a COVID-19 diagnosis in children seeking care in either the outpatient or hospital setting in the Netherlands.
Study Design: Multi-center descriptive prospective cohort study.**Study population:** Children aged 0-18 years diagnosed with COVID-19 in hospitals, and included in the previously approved pediatric study, named "clinical features of COVID-19 in pediatric patients" (also known as COPP), or in the upcoming study "COVID-19 in Pediatric patients: clinical and immunological features (COPP-IMM study; NL76177.058.21). In these studies, the clinical features, course of disease, response to treatment and risk factors for severe disease in hospitalized and outpatient pediatric patients with COVID-19 in the Netherlands, are described.

Main study parameters/endpoints:
Primary endpoints will be

- long-term morbidity (defined as frequency of long-COVID symptoms, any hospital readmission, emergency or outpatient visit for long-COVID pulmonary symptoms, prescribed antibiotics for pulmonary infection since the diagnosis of COVID-19)
- the immunological profile during follow-up at 4-12 months after presenting to Dutch hospitals with COVID-19 or COVID-19 associated Multisystem Inflammatory Syndrome (MIS-C)

Secondary endpoints include

- measures of neurocognitive behavioral and school functioning (patients > 6 years)
- quality of life scores
- exhaled breath profiles
- frequency of pulmonary function test abnormalities, including exercise intolerance
- frequency and pattern of CT-chest abnormalities
- the correlation between immunological profiles at follow-up and detailed clinical parameters.
- prevalence of olfactory dysfunction at long-term follow-up (4 to 12 months) in previously hospitalized children with COVID-19.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: We do not expect individual patients to benefit from the study, other than early detection and, whenever possible, treatment of any abnormalities. The results from this study will benefit the target group, i.e. children with COVID-19 or MIS-Crelated diseaewith, with possible long term morbidity, leading to early detection and hopefully treatment.

All children and/or their caregivers will be asked for consent to withdraw blood for immunological evaluation. The amount of blood drawn depends on the body weight of the patient, and ranges from 5 to 50 ml (see paragraph 8.3). To ensure minimal burden, the collection of the blood sample will be combined with a blood sample collection for routine clinical care if indicated and possible. If this is not possible, venipuncture will be done for study purposes. In this case, there will be some burden to the patient. To ensure that this burden is minimal, we will apply a topical anaesthetic (lidocaine/prilocaine or lidocaine/tetracaine as per local guidelines) and will instruct local researchers on positive language before and during the procedure. If children and/or their caregivers do not consent to the blood withdrawal, participation for the other parts of the follow-up study is still possible.

The main burden of participating in this study, next to blood withdrawal, is visiting the hospital once, undergoing a physical examination, odour identification testing, pulmonary function testing, exercise testing, exhaled breath collection, and completing 5 questionnaires. In patients older than 6 years and visiting the COPP2 study site at AmsterdamUMC a neuropsychological evaluation will be performed during a second study visit. The expected required time-investment of the children and their caregivers is approximately 2-6 hours, depending on the age and willingness of the child. If these tests were already performed at least 3 months after the acute phase of COVID-19 related disease, children and/or their caregivers will be asked for permission to share these results with the investigators.

Depending on symptoms and risk factors for known complications children will be referred to the outpatient clinic for standard care. Caregivers and/or children will be asked for consent to have data from these outpatient visits retrieved from the hospital file and registered at the study CRF. The procedures which are solely part of research are lung function tests (including exercise tolerance), immunological evaluation, exhaled breath analysis, neurocognitive evaluation and an odour identification test.

In case of incidental findings, patients will be referred to their general pediatrician or family doctor for further treatment and follow-up. In case of neurocognitive dysfunction or psychosocial problems (based on questionnaires), patients will be referred to mental health care professionals in their own living area.

To reduce the burden of travelling, patients will be reviewed at the participating study site, closest to their home.

Both the clinical disease-course and the capacity to recover are different in children when compared to adults and we therefore cannot deduce long-term pulmonary sequelae from adults COVID studies.

1. INTRODUCTION AND RATIONALE

Severe Acute Respiratory Syndrome coronavirus (SARS-CoV-2) is currently responsible for a severe pandemic. It was first reported in Wuhan, Hubei province in China in December 2019 (1). In the Netherlands, the first case was reported on February 27th, 2020 (RIVM).

The infection leads to the disease known as Corona Virus Disease 2019 (COVID-19). Clinical features in adults range from mild non-specific respiratory symptoms (e.g. sore throat, cough, fatigue, mucus, myalgia, headache) to a more severe illness (e.g. fever, pneumonia, acute respiratory distress syndrome (ARDS), septic shock and multi-organ failure). In adults, an estimated 80% of infections follow a mild course, 13.8% of patients have severe complaints and 6.1% of patient suffer from very severe disease. In China, the case fatality rate was estimated 2.3%, but the true case fatality rate may be lower if there are more mildly symptomatic cases that are not detected (2-4).

The current data suggest that SARS-CoV-2 infection is less severe in children. The first data, summarized in a systematic review, show that children have accounted for 1-5% of diagnosed COVID-19 cases, have milder symptoms, and death rate is extremely low. Clinical signs are similar to adults, but appear to arise much less frequently (5). In the largest study to date, over 90% of 2.143 children with laboratory verified SARS-CoV-2 were asymptomatic or had mild to moderate disease, 5.2% had severe disease (dyspnea, central cyanosis, oxygen saturation < 92%), and 0.6% had critical disease, needing ICU admittance, because of respiratory failure, ARDS, shock, or multi-organ failure. Those with critical disease had underlying health conditions. Severe disease was mostly seen in children aged less than one year (5-7).

New data in this field are rapidly emerging and data from Spain and the United States of America were published after publication of this review. In Spain, 365 children were screened in 30 hospitals in the first 2 weeks of the pandemic and 11.2% tested positive for SARS-CoV-2 (8). Of those testing positive, the median age was 1 years old (range 0-15 years), 25 (60%) of these children were hospitalized, and 4 were admitted to a pediatric intensive care unit. While interpreting these figures, one should take into account the very restricted testing policy, where only patients with hospital presentations were tested. Initial clinical presentations were upper respiratory tract infection (34%), fever without a source (27%), viral like pneumonia (15%), bronchiolitis (12%), gastro-enteritis or vomiting (5%), bacterial-like pneumonia, (5%) and asthma flare-ups (2%). Two patients had a coinfection with influenza B.

In the US, 2572 children tested positive for SARS-CoV-2. Of these, signs and symptoms were known in 291 children: 56% showed fever, 54% cough, 13% shortness of breath, 23% myalgia, 7% a runny nose, 24% a sore throat, 28% headache, 11% nausea/vomiting, 6% abdominal pain, 13% diarrhea. Among 345 children with information on underlying conditions 23% had at least one underlying condition (of which: 50% asthma, 31% cardiovascular disease, 13% immunosuppression). Six patients were admitted to an ICU and, unfortunately, 3 of them died. Review of these cases is ongoing to confirm COVID-19 as the likely cause of death. 32% of SARS-CoV-19 infections occurred in children aged 15-17, 27% in children 10-14 years, 15`5 in children 5-9 years, 11% in children aged 1-4 year and 15% in children < 1 year (9).

Liguoro published a recent systematic review including the previous articles in which 4780 children with SARS-CoV-2 infection were summarized, confirming the relatively mild disease. In this study 9.2% of children needed oxygen suppletion, and 2% was admitted on the pediatric intensive care unit and required mechanical evaluation. Mortality was 0.08% (10).

During the current pandemic novel scientific information about COVID-19 in children emerges quickly. Although respiratory complaints remain the main reason for admittance, we also see a large group of especially young children with fever as main presenting symptom and a group of young teenagers with a systemic mucocutaneous-enteric illness, or diagnosis of Multisystem Inflammatory Syndrome in Children, (MIS-C, frequently resulting in ICU admittance (11,12)).

MIS-C is a Kawasaki like disease related to SARS-CoV-2 infection. Whittaker describes in a retrospective study 58 children with MIS-C. Their median age was 9 years old (5.7-14), with 33% girls. 26% had a positive PCR for SARS-CoV-2, and 87% had positive IgG. All children presented with fever and nonspecific symptoms, like vomiting (45%), abdominal pain (53%), diarrhea (52%), rash (52%) and conjunctival infection (45%). CRP and ferritin levels were high. 29% developed shock and required inotropic support and fluid resuscitation, of these 79% required mechanical ventilation. 13 of them met the criteria for Kawasaki disease (13).In the Netherlands, 50.147 patients have tested positive for SARS-CoV-19, of which 11.285 were admitted to the hospital, and 5.422 patients have died. Of these 50.147 positive patients, 1.056 (2.3%) were younger than 19 years old, and 91 were admitted to the hospital (RIVM data June 29th, 2020). A survey by the Dutch Pediatric Society showed 10 additional patients who were diagnosed with MIS-C, in the early months of the pandemic in 2020 (14). However, by February 2021, we have already treated around 110 patients with MIS-C in Dutch Hospitals, about 50% was admitted to the paediatric intensive care unit, due to severe cardiogenic shock (data NVK webinar 16-2-2021 and website COPP study https://www.covidkids.nl/scientific-dashboard/ (14)).

Due to lack of sufficient testing material, for a long time we have had a restrictive SARS-CoV-2 testing policy in the Netherlands, so the number of Dutch inhabitants, including children, who were infected with SARS-CoV-2 is probably much higher. Since June 2020, testing is possible for all Dutch inhabitants. Although the incidence of severe or critical COVID-19 disease in children is low, some children do get very sick. Shekerdemian et al. published a case series of 48 patients from Canada and the US who were admitted in a period of three weeks in the pediatric ICU. 83% of them suffered from an underlying condition. 50% had one comorbidity, 17% two and 19% had 3 or more significant comorbidities. 40% was medically complex, defined as children with a long term dependence on technical support associated with developmental delay and/or genetic anomalies. At admission 69% were severely or critically ill, 23% experienced multi-organ failure. 81% required respiratory support that exceeded their baseline (oxygen 13%, HFNC 23%, CPAP 8%, mechanical ventilation or tracheostomy ventilation 38%). No children required renal replacement therapy. It is encouraging that in this cohort only 5% of cases were fatal, compared to adult cohorts in which 50-62% of cases are fatal (15).

Long COVID is the lay term for long term complaints in the period of weeks to months after COVID-19. In scientific literature there is no consensus about the nomenclature yet.(16) In adults, many researchers have expressed their concern about the high frequency of long-term complaints after the active phase of COVID-19, resulting in persistent morbidity, reduced quality of life and inability to participate in family and working life. Recent literature describes a percentage of 10% of patients in the general population.(16) This percentage is higher in cohorts of admitted patients.(17-19) The main complaints in adults are cough, low grade fever, fatigue, shortness of breath, chest pain, headaches, persistent impaired smell and taste, neurocognitive difficulties, muscle pains and weakness, gastro-intestinal complaints, rashes, thromboembolic conditions and depressions. Some have skin rashes like vesicular, maculopapular, urticarial or chilblain like lesions of the extremities. (20)

There is to this date no clear explanation for the occurrence of long-term complaints after the acute phase of COVID-19, but multiple suggestions have been made, such as a weak or absent antibody response, inflammatory reactions, deconditioning or mental factors like stress. (89) In other coronavirus infections (SARS-and MERS) respiratory, neurological, cognitive, and musculoskeletal sequelae have also been described. (21,22)

In the Netherlands this has led to a diagnostic and treatment plan for adults with long COVID, in collaboration with several medical associations, resulting in clear diagnostic and treatment tools. In the last couple of months multiple developments has led us to believe that “long COVID” in children deserves more attention than it currently gets. In scientific literature, case series of small cohorts of pediatric patients with complaints of long COVID are emerging. Our own research group performed a survey among Dutch pediatricians, revealing 89 cases of children, suspected of long COVID, treated by Dutch pediatrician. All were not admitted in the hospital in the acute phase of COVID-19, but were suffering from long term complaints, like fatigue (87%), dyspnea (55%), concentration difficulties (45%), headaches (38%), thoracic pain complaints (35%), stomach ache (33%). 36% experienced severe limitations in daily functioning, like less or no school attendance. We suspect that these children are only the tip of the iceberg since some children with long-COVID may only be treated by the general practitioner. Furthermore, long-COVID is still an unknown phenomenon to many pediatricians likely resulting in underdiagnosing. (23) In a single-center study from Italy by Buonsenso et al., persistent symptoms in children previously diagnosed with COVID-19 were reported in 42.6% of children. (24) The reported long-COVID symptoms in this Italian study showed resemblance to the symptoms reported in our survey. In the United Kingdom, long term complaints in children, not admitted to the hospital, were reported 5-12 weeks after a positive test for COVID-19 in 12-14% of all patients.(25) Furthermore, one case series describes 5 children with a median age of 12 years (range 9-15), with symptoms 6-8 months after their clinical diagnosis of COVID-19. (26) In newspaper publications many more of these patients have been described. (27,28) Also, several colleagues and parents have reached out to our research group with their experience with patients/children with a history of COVID-19 and persistent complaints of exercise intolerance, persistent coughing, tiredness, chest tightness, palpitations, difficulties with concentration, memory, performing multiple tasks, and a reduced ability to participate in daily life.

The COPP and COPP-IMM study are two large multicenter, observational, prospective cohort studies in hospital-setting in the Netherlands, on pediatric COVID-19 and MIS-C. (11) Currently, 53 of the 72 pediatric departments in the Netherlands are participating in COPP and 13 out of 72 will participate in COPP-IMM. From an international view this is an unique study in which national collaboration of most pediatric departments provides an excellent overview of the impact of COVID-19 in pediatric care. The COPP study and COPP-IMM study aim to include all pediatric cases in hospitals in the Netherlands, and collaborates closely with the Dutch Pediatric Society and researchers from all seven University Medical Centers in the Netherlands.

For COPP2, we aim to include all children with COVID-19 who were diagnosed in the outpatient department or were hospitalized, and who were included in the COPP study or COPP-IMM study, in which their clinical features were described.

In summary, next to follow-up of pulmonary sequela and quality of life, we will also investigate neurocognitive and olfactory morbidity, and the long-term immunological response in all patients. Depending on symptoms and risk factors for known complications these children, will be referred to the outpatient clinic for standard care.

Caregivers and/or children will be asked for consent to have data from these outpatient visits retrieved from the hospital file and registered at the study CRF. The procedures which are solely part of research are lung function tests (including exercise tolerance), immunological evaluation, exhaled breath analysis, neurocognitive evaluation and an odour identification test.

In the following sections we will describe in detail the rational for the investigations.

1.1 Pulmonary involvement.

COVID-19 is a new disease, therefore, in terms of choice of follow-up investigations, we have to rely on our experience with other viral pneumonia's or bronchiolitis patients and the established CT abnormalities in COVID-19 patients. Since SARS-CoV2 is one of the coronaviruses, we sought evidence for the long term effects in children who had suffered from severe coronaviruses in general. SARS-CoV-1 shows some similarities to SARS-CoV-2 and also runs a more benign course in children. Li et al described 47 patients (median age 13.6 years) who had a history of hospital admittance due to severe acute respiratory syndrome (SARS-CoV-1). All children were asymptomatic and had no physical abnormalities at 6 months follow-up. However, mild pulmonary abnormalities were detected on HRCT in 34% of these patients, including residual ground-glass opacification, air trapping, and a combination of these. There were no lung function abnormalities, but exercise testing showed exercise impairment in these patients, with a reduced peak oxygen consumption compared to controls. At 15 months of follow-up, these patients still showed lower absolute and mass related peak oxygen consumption, higher ventilatory equivalent for oxygen, lower oxygen pulse and a lower oxygen uptake efficiency slope. Chest CT abnormalities at 15 months did not differ from the CT scan results at 6 months follow-up (29-31).

Of the 674 children with SARS-COV-19 infection in the study by Liguoro who had radiological examination, up to 49.1% showed abnormalities, even if asymptomatic (15% CT abnormalities). 73.9% underwent a chest CT scan, which was normal in 32.7% of cases, whereas typical groud-

glass opacities, nonspecific unilateral and bilateral lesions were identified in 29.4, 26.6 and 23.2% of patients, respectively (10).

Cui et al investigated specifically CT abnormalities in children and included 2597 patients in a systematic review (many of these studies were also included in Liguoro's review). 7.6% were asymptomatic, 45.5% and 41.5% had mild or moderate disease, 4.4% had severe disease, and 0.9% were critical. From 409 patients chest CT results during time of admittance were known, 43.5% had normal CT scans, in the other patients ground-glass opacities, local patchy or bilateral shadows, interstitial lesions or "white lung change" were seen (32).

In a Chinese cohort with adult COVID-19 patients with a median age of 43 years, 53% showed complete radiological resolution at three weeks postdischarge. The predominant pattern of abnormality observed at discharge were ground-glass opacification (44%), fibrosis (30%), and thickening of the adjacent pleura (10.7%). In this study, all patients suffered from pneumonia, and no critical patients were included (33). Bernheim et al showed that in adult patients with severe COVID-19 CT abnormalities showed progression during the course of disease, with 28% of patients with bilateral lung involvement in the early phase of disease (0-2 days) and 88% at a late stage (6-12 days) of disease. In this study no long term follow-up data were available. There are no follow-up CT data in children with COVID-19. (34)

Summarized, ground-glass opacification, pulmonary fibrosis are the most common CT abnormalities in patients with COVID-19. 34% of children with a history of SARS-COV-1 show CT abnormalities at 6 months follow-up, despite lack of clinical symptoms.

HRCT and lung function tests are the investigations of choice for the detection and evaluation of air-space and airways diseases, especially if associated with small airways disease. Ground glass opacifications and fibrosis, found in adults with COVID-19, may lead to restrictive lung disease. However, in children, especially the younger group, a clinical diagnosis of bronchiolitis or bronchitis is also seen in relation to SARS-CoV-19 infection. Most children with other causes of viral bronchiolitis or pneumonia have a short course of disease, however, some suffer from post-infectious bronchiolitis obliterans, an obstructive lung disease.

In the current Dutch protocol for children with severe COVID-19 CT scans currently are not performed, since CT abnormalities in children are not specific for COVID-19 (35). In the Dutch proposal for the protocol concerning follow-up of adult COVID-19 patients CT scans are performed in case a patient shows abnormal pulmonary function tests or do have respiratory complaints.

In this study we use questionnaires about respiratory symptoms and quality of life, pulmonary function tests and exhaled breath profiles to determine pulmonary morbidity in the follow-up of COVID-19 in children, if needed. We expect to be able to identify restrictive or obstructive pulmonary disease, and diminished diffusion capacity, leading to reduced exercise tolerance. HRCT in patients without current symptoms was found not ethical. Therefore, we will only perform CT scan in patients with current respiratory symptoms or pulmonary function test abnormalities, in which there is a clinical need for further evaluation.

1.2 Persistent olfactory dysfunction.

Smell and taste loss is common in patients with COVID-19 (37). As of May 13, 2020, the European Centre for Disease Prevention and Control, the World Health Organization and several countries, including The Netherlands, have listed smell loss as a typical symptom of COVID-19.(36,37)

SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) receptors, which are highly expressed in nasal epithelial cells, as its main receptor to enter the human body. SARS-CoV-2 seems to infect peripheral support cells, consequently affecting olfactory sensory neurons.(38) Recent data suggest that SARS-CoV-2 also independently affects taste and chemesthesis.(39) This mechanism is less clear, however, subjectively experienced taste loss can also be attributed to impaired retronasal olfaction (flavor) rather than impaired gustation (sweet, salty, sour, bitter, umami). Currently, it is thought that both reported smell and taste loss in COVID-19 can primarily be ascribed to impaired olfactory function.

Prevalence rates of olfactory dysfunction range between 59% and 86% in COVID-19 non-hospitalized adult patients. (40-42) Sometimes, smell loss is the only manifest symptom. (43) Olfactory dysfunction in COVID-19 hospitalized adult patients has been consistently reported at lower incidence rates (5-35%).(44,45) It is not completely clear if critically ill patients experience less smell and taste loss than patients with mild/moderate disease do. (46) Probably, they are less aware about smell and taste loss due to the presence of more severe disease-related symptoms.

In children, data on the prevalence or severity of smell and taste loss during COVID-19 are scarce. Most evidence comes from small case reports and medical records of adolescents.(47,48) For example, a retrospective international multicenter study, including 27 hospitalized children, found self-reported olfactory or gustatory dysfunction among 10 of them (37%, age range: 15 – 17 years).(50) Another observational study among 2463 Canadian non-hospitalized children reported smell and/or taste loss as the strongest predictor of a positive SARS-CoV-2 swab result (positive LR 7.33, 95% CI 3.03–17.76).(49) Until now, only one study has objectively investigated smell function in children with COVID-19 by a novel olfactory screening test that was designed to easily and safely measure olfaction during the pandemic.(50) One month after infection, subtle disturbances (misrecognition of odors) were still identified among the infected children. It remains unclear to what extent children hospitalized with COVID-19 or MIS-C experience smell and taste loss during admission and on long-term follow-up. In approximately 10% of adult patients, smell and taste loss seem to persist 1.5 – 6 months after COVID-19 infection.(51) The burden of these long-term symptoms can lead to a severe disruption of daily life, including psychological well-being and health.(81) Based on these data we believe that assessment of the prevalence of olfactory dysfunction during follow-up, and its possible relationship with quality of life in pediatric patients with COVID-19 is needed.

1.3 **Neurocognitive evaluation:**

In people suffering from COVID-19, the brain can be impacted in multiple ways, both directly and indirectly. Direct brain infection by SARS-CoV-2 may occur via axonal transport via the olfactory nerve, or hematogenous, which involves viral crossing of blood-brain barrier. Secondary mechanisms involve hypoxia, impaired cerebral perfusion in case of cardiogenic shock, and an aberrant immune response leading to various forms of encephalopathy, white

matter damage, and abnormal blood clotting resulting in stroke. Emerging evidence from adult studies suggests that 36.4% of COVID-19 patients exhibit neurological symptoms including both central and peripheral signs. (52) Central signs include consciousness-impairment, vomiting, headache, dizziness and nausea, whilst the peripheral signs include different types of hypoesthesia suggesting central nervous system-invading capabilities of the virus where it may affect functioning of specific nuclei or neural circuits. Apparently, among these neurological manifestations, those presenting early and those presenting later in the course of the COVID-19 pathology can be identified. In adults with mild to moderate COVID-19, sustained sub-clinical cognitive impairment was reported in 14 of 18 young adults.(52) In a cohort of 35 adult patients, 34% had cognitive complaints. Patients presenting with headache, anosmia, dysgeusia, diarrhea and those who required oxygen therapy had lower scores in memory, attention and executive function subtests as compared to asymptomatic patients. Patients with headache and clinical hypoxia scored lower in the global Cognitive Index (53). Case series and cohort studies, concerning long COVID showed a high percentage of children suffering from fatigue, difficulties concentrating and memory loss.(23-26) All of these children are missing school because of their symptoms. It is suggested that acute physiopathogenetic mechanisms through which also SARS-CoV-2 affects the CNS can now be identified during the acute phase of COVID-19: direct viral encephalitis, systemic inflammation, peripherical organ dysfunction (liver, kidney, lung) and cerebrovascular changes.(54) In the long-term perspective, one or more of these mechanisms together may contribute to raise the risk for developing long-term neurological complications in COVID-19 patients. This assumption has been confirmed by the observation that about one third of COVID-19 adult patients discharged have cognitive problems. However, this assumption has not yet been confirmed in children – other than that clinical observations show long-term complaints of fatigue and difficulties concentrating, weeks to months after the acute phase of COVID-19.

1.4 Immunological evaluation

Although in general children are less affected by COVID-19, a subset of children with COVID-19 develops severe disease (55-58).(55-58). In adults, severe COVID-19 is characterized by a hyperinflammatory response and cytokine storm. Typically, this hyperinflammatory response does not occur when children are infected with SARS-CoV-2. However, a subset of children is admitted to hospital with COVID-19, and a subset requires supplemental oxygen or even intensive care. In rare cases, children develop MIS-C, a post-infectious hyperinflammatory syndrome some weeks after infection with SARS-CoV-2. (59) MIS_C is considered to be part of the disease spectrum of COVID-19 in children.(59) There are marked differences between the immunological profiles of children with MIS-C and adults with severe COVID-19 compared to adults (60). However, much less is known about the immunological profile of children with severe COVID-19. and how this relates to the immunological profile of children developing MIS-C. Also, some children suffer from long-COVID. In adults, prolonged inflammatory responses after a SARS-CoV-2 infection are detected and hypothesized to be responsible for longpost-acute sequelae of COVID. -19.There is to date no etiological research on long COVID complaints in children.

In the COPP-study we aim to describe which children are at the highest risk of developing severe COVID-19 or MIS-C and what the exact course of illness is in these children. In the COPP-

IMM study (under evaluation of the METC-LDD, NL76177.058.21), we will correlate these detailed clinical findings with a comprehensive and integral analysis of the innate and adaptive immune response of children in Dutch hospitals with COVID-19 or MIS-C. In the immunological substudy of the current COPP2 study, we will perform a comprehensive analysis of the immune response in children months after the acute phase of COVID-19 or MIS-C. This will enable us to study the quality and longevity of the humoral and cellular response against SARS-CoV-2 in children months after the initial infection. Also, it will enable a longitudinal analysis of cytokines in those children with a hyperinflammatory profile at diagnosis, and we can relate possible long term sequelae to the immunological profile.

1.5. Retrieval of medical data from patient files:

Patients with long term complaints after a diagnosis of COVID-19 related disease are in need for clinical evaluation, depending on their symptoms. Data regarding standard of care evaluation from patients included at COPP2 will be retrieved from the hospital files, after signed consent from parents/guardians and/or patients. This will include cardiac evaluation (cardiac ultrasound, ECG, cardiac MRI, cardiac markers), hematologic evaluation for post thrombotic sequelae (compression ultrasound and trombotic markers),nephrological evaluation (nephrological markers in blood and urine) and neuropsychological follow-up.

2. OBJECTIVES

Primary objectives:

- To asses sequelae of COVID-19at 4 to 12-months following a COVID-19 diagnosis among pediatric patients receiving care in the hospital or outpatient setting in the Netherlands.
- To determine risk factors for long-COVID among COVID-19 in- and outpatient pediatric patients in the Netherlands.
- To obtain a detailed immunological profile of children at 4-12 months of follow-up after presenting to Dutch hospitals with COVID-19 or with SARS-CoV-2 related post-infectious inflammatory syndrome (MIS-C).

Secondary objectives

- To describe pulmonary function testing abnormalities in the follow-up of children with COVID-19.
- To describe exhaled breath profiles (SpiroNose/GC-MS) in children with a history of COVID-19.
- To describe CT abnormalities in patients with chronic respiratory complaints and/or pulmonary function test abnormalities and a history of COVID-19.
- To assess the quality of life in all children with a history of COVID-19.
- To assess parental perception of cognitive functioning and executive functioning in all children with a history of COVID-19.
- To assess neurocognitive functioning in a consecutive sample of children with a history of COVID-19 (aged 6 or older).
- To correlate the immunological profiles with detailed clinical parameters
- To evaluate the cellular and humoral immunity to SARS-CoV-2
- To determine if there is a difference in the long-term cellular and humoral immunity to SARS-CoV-2 in children with COVID-19 related disease.
- To describe prevalence of olfactory dysfunction in children with a history of COVID-19.

3. STUDY DESIGN

Study design: This will be a multi-center, descriptive observational prospective cohort study.

Duration: Following an initial baseline registration as part of the COPP study or the COPP-IMM study, children and their caregivers will be requested to return for a follow-up visit at 4 to 12 months after COVID-19 diagnosis. We will enroll participants throughout a 1.5 year period.

Setting: Pediatric department. Children previously included at the COPP or the COPP-IMM study who provided consent to be approached for a possible participation in the COPP2 study.Children from both the COPP and COPP-IMM study will be asked to participate in the COPP2 study. (16) All children will be asked to come to the hospital for their follow-up visit for the COPP 2 study, at 4 to 12 months after diagnosis with COVID-19.

Description: Children aged 0-17 years who were diagnosed with COVID-19 will be recruited from the COPP or COPP-IMM , if they gave permission to be approached for follow-up studies. Study measurements include: questionnaires and physical examination, growth measurements and immunological profile for all children and exhaled breath (SpiroNose/GC-MS), pulmonary function, exercise testing, odour identification testing and Chest CT scans in a subgroup of patients. Patient data regarding clinical care will be retrieved from patient files after receiving informed consent from parents/guardians and/or children.

Figure1. Flow-chart for study inclusion and procedures

COPP and COPP-IMM study: Permission to approach for follow-up study? → COPP2 study

COPP2 study: Long-term follow-up of patients with COVID-19: multi center, prospective descriptive cohort study.				
All 3 study centers:	All ages: symptom questionnaire Quality of life questionnaire: >0-2y: TAPQL >2 y: PedSQL >8y: + PROMIS Screening neurocognitive and executive functioning: >6y: BRIEF + PedsPCF	All children > 2 y: exhaled breath analysis AND All children > 4y: spirometry AND All children > 6 y: odour identification testing, exercise testing AND All children > 8 y: body Plethysmography	All ages: Blood withdrawal*	Procedures during COPP2 study visit
AmsterdamUMC	>6 y: Neurocognitive screening and assessment: Emma Toolbox			
All ages:	Retrieval of information from patient files, concerning: -Chest CT scan - hematological evaluation of post trombotic sequelae - nephrological evaluation - cardiac evaluation - PICU follow-up evaluation (including neurocognitive assessment)			Retrieval from patient file

*_This is an (mildly) invasive procedure: individual opt-out is possible.

4. STUDY POPULATION

4.1 Population (base)

Pediatric patients with COVID-19or MIS-C who presented at the emergency or outpatient department and/or were hospitalized in a hospital in the Netherlands. From the beginning of the pandemic until March 2021, 180.000 children and adolescents less than 20 years of age have been tested positive for SARS-CoV-2COVID-19 in the Netherlands (source: RIVM weekly update march 23rd 2021). In this same period, 206 children (march 23rd, 2021) have been included in our prospective observational cohort study, the COPP study. The COPP-IMM study has yet to start after approval of METC LUMC. Depending on the course of the COVID-19 pandemic, ongoing public health measures, the course of the “third wave”,” and any subsequent “waves”, and the availability of a vaccine available for children, the number of pediatric cases of COVID-19 will rise. Therefore, we expect to be able to include a considerable number of pediatric patients over the course of 1.5 year (June 2020-December 2021), with a maximum of 120 patients.

4.2 Inclusion criteria

A child will be eligible for this study, if he/she was previously enrolled in the COPP-study or COPP-IMM study and gave specific consent to be approached for follow-up studies.

Inclusion criteria for COPP/COPP-IMM are:

- Aged 0-17 years at COVID-19 diagnosis, AND
- Presented to an emergency or outpatient department of a Dutch hospital and/or admitted to hospital, AND

AND

- Have at least one positive real-time RT-PCR test on nasopharyngeal, oropharyngeal, sputum or fecal sample for SARS-CoV-2 OR
- Proof of a (recent) infection with SARS-CoV-2 by positive serology test (IgG/IgM) OR
- Fulfil a clinical diagnosis (*) of COVID-19, should testing of SARS-CoV-2 yield inconclusive results and/or if testing is no longer possible due to lack of reagents.

(*) This includes:

- signs, symptoms and imaging results indicative of COVID-19 pneumonia without evidence for other causes; OR
- inflammatory syndrome with a history consistent with recent COVID-19 AND/OR laboratory results consistent with current COVID-19 OR recent COVID-19 (e.g. positive SARS-CoV2-PCR and/or positive serology).

4.3 Exclusion criteria

A child will be excluded from the study, if:

- Consent from guardians and/or patient is not received, or
- Consent for COPP/COPP-IMM study data is not received.

4.4 Sample size calculation.

Not applicable. We will include all patients who were included in the COPP study in the course of 18 months, with a maximum of 120 patients.

As this is an observational cohort study a clear power calculation is not possible. Previous studies in SARS showed that 16/47 children (34%) who were treated in hospital in Hong Kong had abnormal chest CT's 6 months post-diagnosis (9-11). In addition, 11% of children who were able to perform spirometry (n=38) had lung function abnormalities. There are currently no data on olfactory function, taste or neurocognitive follow-up in children with COVID-19. In the current COPP cohort a very wide spectrum of patients with COVID-19 is included, ranging from two-day-old neonates to 17-year old children, with different medical backgrounds and different presenting symptoms of COVID-19. Based on these observations, we plan to perform an interim analysis for the more burdensome and time consuming measurements that are related to clinical care. (paragraph 10.4)

5. TREATMENT OF SUBJECTS

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

- long-term 'morbidity' or sequelae, defined as: Frequency of persisting symptoms, hospital readmission, emergency department or GP visits for persisting symptoms, antibiotic courses for infections or start of inhaled corticosteroids and/or b2-mimetics after admission for COVID-19 since diagnosis of COVID-19;
- The immunological profile of children at 4-12 months of follow-up after presenting to Dutch hospitals with COVID-19 related disease.

8.1.2 Secondary study parameters/endpoints (if applicable)

- Quality of life score in all children and frequency of abnormalities in the neurocognitive profile of patient older than 6 years of age.
- Growth.
- Frequency of pulmonary function tests abnormalities, including exercise intolerance.
- Exhaled breath profiles (SpiroNose/GC-MS).
- Frequency and pattern of Chest CT abnormalities in patients with chronic pulmonary complaints and/or pulmonary function abnormalities.
- The longevity and quality of the humoral and cellular adaptive immune response in children with a history of COVID-19.
- Complete normalization of hyperinflammatory cytokine profiles in children with COVID-19 after the infection with SARS-CoV-2
- Correlation between the immune response and cytokine profiles and long COVID complaints.
- Prevalence of olfactory dysfunction at long-term follow-up (4 to 12 months) in previously hospitalized children with COVID-19.

8.1.3 Other study parameters

Not applicable

8.2. Randomisation, blinding and treatment allocation

The neurocognitive assessment battery and the assessment of exhaled breath patterns via Spironose will only be administered in patients visiting the Amsterdam University Medical Centers (this due to issues of patient (time) burden, logistics and finances)

8.3 Study procedures

Patients will be included following a SARS-CoV-2 positive test result (or fulfilling a clinical diagnosis of COVID-19) and/or clinical signs of COVID-19 in their medical history, enrollment in the COPP study or COPP-IMM study, combined with informed consent (IC) for contacting patient and/or guardians for a follow-up study, and after obtaining IC from guardians and/or patients.

Clinical data at the time of (in-hospital) treatment of COVID-19 are noted in the database shared by the COPP study and COPP-IMM study. (see appendix I). These will be combined with follow-up data from the COPP2 study, consisting of the following:

Questionnaires about complaints:
A questionnaire regarding symptoms will be used to assess complaints 6 months after diagnosis, this contains questions about use of healthcare facilities, given therapy, and general, pulmonary, cardiac, hematological, and smell or taste difficulties. Furthermore, we will ask for a history of central venous catheter, cardio vascular disease, renal conditions. (see F1 documents).

Physical examination and height/weight.
All children will undergo physical examination, including evaluation of ear - nose – throat, pulmonary and cardiac abnormalities during chest examination, vital signs (including measurement of blood pressure as described in SOP blood pressure measurement) and measurement of height and weight.

Quality of life questionnaires:
We will use the PedSQL questionnaire in all children aged 2-18 years old (proxy report for children < 8 years old), the TAPQOL questionnaire for children 0-2 years old (proxy report), and the PROMIS questionnaire (self-report) in patients older than 8 years of age. Combined, these questionnaire will take approximately 10 minutes to complete.

The PedsQL and TAPQOL questionnaires measure Health Related Quality of Life and allow a comparison between HRQOL at 6 weeks and 6 to 12 months after diagnosis in all children. The PedsQL and TAPQOL are well translated, validated questionnaires and Dutch norm scores are available.

To measure overall health and specific domains of functioning (anxiety, depression, peer relationships, sleep problems, anger, and neurocognitive functioning), we will use the PROMIS items in children older than 8 years of age. During the present pandemic, norm data for these items are currently being collected. Including these PROMIS item banks provides the opportunity to compare outcomes of children with a history of COVID-19 to their healthy

counterparts with the same restrictions. Comparison of the quality-of-life scores at 6 weeks and 6 to 12 months after diagnosis is possible.

Neurocognitive evaluation

Parents of patients > 6 years are asked to fill out questionnaires that are designed to assess parental perception of cognitive (Peds-PCF) and executive functioning (BRIEF). Combined these questionnaires will take approximately 15-20 minutes to complete.

All children > 6 years, assigned to Amsterdam University Medical Centers for their study visit, will undergo neurocognitive assessment as part of the second study visit, by a well -trained neuropsychologist (using the Emma Toolbox) if this has not been performed previously as part of standard care. This assessment is needed to evaluate whether the screening questionnaires used (BRIEF and Peds-PCF) are suitable for detecting abnormalities in children with long COVID complaints.

Screening measures: questionnaires (online: www.hetklikt.nu)			Duration
Peds-PCF Short Form (parent report)	Perceived Cognitive Functioning	7-items	2 minutes
BRIEF (parent report)	Different aspects of executive functioning	86-items	10-15 minutes

AmsterdamUMC only: extensive neurocognitive assessment in children > 6 years old

The Emma Toolbox:

Task	Cognitive domain	Description	Duration
WISC-V[NL] (short form, using digital tools) (child)	Intelligence	Similarities, Vocabulary, Matrix Reasoning, Block Design, Digit Span	30 min
Emma Toolbox for neurocognitive testing (ETB) (child)	Information processing, attention, learning & memory, executive functioning and sensory & motor functioning	Battery of computerized tests	75 min
SWAN (parent, teacher)	Attentional functioning	18 items questionnaire	5 min
SDQ (parent, teacher, self-report if child > 11 yrs of age)	Behavioral functioning	25 items	5 min
CITO pupil monitoring system	School functioning	Existing data gathered at child's school (after permission is obtained by parents/caretakers	-
WAN (parent, teacher)	Attentional functioning	18 items questionnaire	5 min
SDQ (parent, teacher, self- report if child > 11 yrs ofage)	Behavioral functioning	25 items	5 min
CITO pupil monitoring system	School functioning	Existing data gathered at child'sschool (after permission is obtained by parents/caretakers	-

Intelligence will be assessed using the Fifth version of the Wechsler Intelligence Scale for Children (61). Full-scale IQ (FSIQ) will be estimated using the subtests Vocabulary, Similarities, Matrix Reasoning and Block Design, with adequate validity and reliability in estimating FSIQ (61-64). Administration takes approximately 30 minutes.

Specific neurocognitive functions will be measured using the Emma Toolbox for Neurocognitive Functioning. The Emma Toolbox for neurocognitive testing (65,66) is an in-house designed collection of computerized tests that assesses the following neurocognitive domains: information processing, attention, learning & memory, executive functioning and sensory & motor functioning. The Emma Toolbox combines validated and experimental tests, allowing outcome measurement as well as exploration of the etiology of neurocognitive impairment. To

these ends, the Emma Toolbox uses built-in control conditions and parametric difficulty manipulation to isolate the effects of neuropathology on specific neurocognitive processes. The broad range of neurocognitive domains covered by the Toolbox also allows neurocognitive profiling of patient groups. Lastly, the Emma Toolbox is designed to facilitate advanced analysis of task performance based on intra-individual data distributions (e.g. Ex-Gaussian analysis, Diffusion Model analysis), allowing the assessment of aspects of information processing (e.g. strategy, efficiency of the neurocognitive process and response execution) or intra-individual variability (i.e. lapses of attention, a hallmark feature of inattentive and impulsive behavior). Taken together, the Emma Toolbox is an innovative neurocognitive battery to perform an in-depth study on neurocognitive functioning in children, with strong relevance for daily life functioning. The Emma Toolbox is administered on a 15-inch laptop from a 50 cm viewing distance and has a duration of 75 min. All tests are presented as games, therefore children are typically enthusiastic to complete the Emma Toolbox.

Behavioral functioning of children and youth will be assessed using the validated Strengths and Difficulties Questionnaire (SDQ) (67), a widely used test with good concurrent validity (i.e. correlation of SDQ scores with other measures of psychopathology) and reasonable reliability (67). The SDQ assesses the psychosocial adjustment of children and youth. The first part of the SDQ consists 25 items divided over five scales including, emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behavior (67). In the first part scoring is based on a 3-point scale ranging from ‘not true’ to ‘entirely true’. The second part of the SDQ questionnaire assesses difficulties related to emotions, concentration, behavior and within the social environment. Administration takes approximately 5 minutes.

Besides the SDQ, the Strengths and Weaknesses of ADHD symptoms and Normal behavior scale (SWAN,(68,69) will be used to assess behavioral functioning. In particular, this instrument focusses on the assessment of attention skills based on the attention deficit hyperactivity disorder (ADHD) criteria stated in the Diagnostic and Statistical Manual of Mental Disorders (DSM), 4th edition (American Psychiatric Association, 1994). The instrument consists of 9 DSM-IV items for attention deficit and nine DSM-IV items for hyperactivity/impulsivity. Scoring of the items is based on a 7-point Likert scale ranging from ‘far below average’ to ‘far above average’. Thus, SWAN scores cover both the strengths as the weaknesses of a participant. Administration takes approximately 5 minutes.

Dependent on explicit informed consent from the legal guardian, the school performance from the participant will be collected using tests that are part of a pupil monitoring system developed by the Dutch National institute for Educational Measurement (70). These tests are used to assess children’s academic performance in grade one to grade six inI 90% of the primary schools in the Netherlands. Reading comprehension, spelling and arithmetic performance are assesses using standardized tests (71-73).

Pulmonary function testing

Spirometry

Spirometry will be performed in children older than 4 years of age at time of the study visit to measure:FVC: maximal volume of air exhaled with maximally forced expiratory effort from a position of maximal inspiration;FEV1: volume of air expressed in liters exhaled during the first

second of performance of the FVC;bronchodilator reversibility: improvement in the percentage of the predicted normal value FEV1 after administration of 400 mcg of salbutamol.

Spirometry measurements will be performed according to the ATS/ERS 2005 guideline (74). Predicted normal values will be calculated according to GLI (Global Lung Initiative) standards. FVC and FEV1 will be assessed using standardized spirometry equipment, with mouthpieces suitable for the pediatric population. Airflow reversibility will include a pre-salbutamol spirometry, followed by administration of salbutamol by oral inhalation (100 mcg x 4 puffs). Spirometry will be performed 20-30 min post-salbutamol. The reversibility test will be considered positive if patients show improvement of FEV1 ≥ 12% after administration of salbutamol. Spirometry will be conducted while the patient is in a seated position. The test will be done in triplicate (three curves to be provided), and the best result selected according to the guidelines. The best of three efforts will be defined as the highest FVC, obtained on any of the three blows meeting the ATS/ERS criteria with a maximum of five maneuvers. On the day of the study visit, patients must refrain from strenuous activity at least 12 hours prior to pulmonary function testing. Smoking (if applicable) should be discouraged throughout the visit day and will not be permitted in the 30-minute period prior to spirometry. Patients should also avoid cold temperatures, environmental smoke, dust, or areas with strong odors (e.g. perfumes). If treated with bronchodilators, a wash-out of 24 hours for long acting and 8 hours for short acting bronchodilators should be observed before spirometry, respectively.

Body plethysmography
In children older than 8 years of age, plethysmography will be conducted to measure the total lung capacity (TLC), residual Volume (RV) and to determine the specific airway conductance (sGaw) at time of the study visit.

Body plethysmography contains a series of gentle breaths at a frequency between 0.5 and 1.0 Hz to calculate lung volumes. Lung volumes will be displayed as predicted percentage of RV (RV%pred) and TLC (TLC%pred) and RV/TLC.

Pulmonary function tests will be conducted by highly trained professionals with experience performing these tests in pediatric patients. They will ensure that the tests are performed with the correct technique, manually deselect efforts which do not meet minimum standards.

Spirometry and body plethysmography results will be reviewed by two independent pediatric pulmonologists to determine whether abnormalities are present.

Exercise tolerance testing:
Standardized cardiopulmonary exercise testing by cycle ergometry will be performed using the Cooper protocol from the age of 6 years old. (75)

The integrated response of different physiological systems (the cardiovascular, pulmonary, neuromuscular, musculoskeletal, and metabolic systems) will be objectively evaluated at rest, during progressive exercise up to maximal exertion, and during recovery.

Chest Computed Tomography (CT) thorax:
The previous investigations may lead to a clinical indication to perform a chest CT scan. Results of these CT scans can lead to an increased understanding of the pathophysiology of pulmonary sequelae in pediatric patients with COVID-19.

In order to optimize standardization of these CT results, we invite patients to perform the chest CT scan, when indicated, at the hospital where the study visit has taken place.

A Chest CT will only be performed as part of standard clinical care, following standards from both international and national societies (the European Respiratory Society and the Dutch Pediatric Pulmonology Society). In the following cases the investigator will contact the treating physician of the patient to discuss the indication to perform a chest CT scan.

- Chronic respiratory complaints consisting of:
    - Chronic wet cough, not caused by any pre-existing chronic disease
    - two episodes of pneumonia in the 12 months before the study visit. (not all children with COVID-19 have suffered from pneumonia). In children with 2 or more periods of pneumonia in one year chest CT is standard clinical care in the search for underlying disease.
    - Severe exercise intolerance, diagnosed either by exercise testing or by observation by parents. In children < 6 years old and unable to perform exercise testing, we will ask for exercise intolerance observed by parents. A chest CT scan evaluates bronchiectasis, post-infectious bronchiolitis obliterans, pulmonary fibrosis, pulmonary ground glass abnormalities.
- Pulmonary function test abnormalities, not primarily caused by any pre-existing chronic disease. When pulmonary function tests are previously performed, we ask for consent to review previous tests and compare pulmonary function testing results before and after complaints of COVID-19.
    - Not reversible airway obstruction: FEV1 < 80% or FEV1/FVC < 70% post bronchodilation
    - Body plethysmography showing pulmonary restriction or airtrapping: TLC < 80%. RV/TLC >30%.
    - Exercise capacity <80% of normal for age and gender, defined as a VO2 max lower than 80% of normal.

Afterwards we will contact the patient for permission to perform the chest CT scan at the study center. Chest CT's will be performed on a dedicated pediatric scanner, according to the pediatric HRCT protocol. This CT will be performed when the patient is without clinical symptoms of pneumonia.

The Chest CT protocol consists of a CT without IV contrast, performed during the inspiratory phase. When children can follow instructions, an expiratory phase will be added. In children who are unable to follow instructions, a free breathing scan will be performed. The CT with administration of IV contrast might be indicated and will only be performed following clinical symptoms and as a standard of care procedure, as previously described

Anesthesia is not needed to perform the CT scan; young children will be held by a vacuum mattress, which holds them still, while being comfortable. The scan takes about 1 minute to perform, with preparation and explanation approximately 10 minutes in total time. Chest CTs will be scored by two independent radiologists, blinded to the outcome of the patient (see appendix II)

In the event of incidental findings, patients will be referred to their general pediatrician or pediatric pulmonologist.

In case a chest CT scan is already performed as part of clinical care, patients and/or parents approval will be asked for consent to share these CT images with the investigator.

In case a chest CT is performed during the initial admittance, we ask for approval to review these images.

Exhaled breath analysis:
The analysis of volatile organic compounds (VOC's) in exhaled breath (breathomics) is an attractive non-invasive technique that can be used to assess pulmonary phenotypes, which might be implemented in clinical care. Studies of our own research group, as well as that of others, have shown that the collection of exhaled breath is acceptable and feasible in children from the age of 2 years (76). VOC analysis captures gaseous molecules from exhaled air. The two main approaches to study VOCs are 1) gas chromatography – mass spectrometry (GC-MS) and 2) electronic nose (eNose using a SpiroNose) technology. GC-MS enables identification of individual VOC's and consists of 2 parts; gas chromatography, which can separate compounds in a complex gas mixture, and mass spectrometry, to identify the different individual compounds present in the sample. On the other hand, eNose technologies identify complex mixtures of VOC's based on pattern recognition algorithms. The technique consists of cross-reactive nonspecific sensor arrays that are exposed to the VOC's and generate a so-called "breathprint"of the VOC profile. (77,78).

Previous studies have shown that VOC measurements are associated with clinical outcomes such as sputum eosinophilia and sputum neutrophilia in adult patients with chronic respiratory diseases, and can predict loss of asthma control as well as steroid responsiveness in asthma (79-95). However, very limited data is available in children. There are no data available in COVID-19 patients. More research is needed to validate VOC analyses for clinical use in these children and to assess the diagnostic value of a point of care eNose test for the screening for long-term pulmonary abnormalities in COVID-19 patients.

In its current form, the SpiroNose is a spirometry coupled electronic nose based on metal-oxide semiconductor (MOS) sensors with high between-sensor reproducibility. The SpiroNose consists of eight sensor arrays of metal oxide sensors (Breathomix, The Netherlands). Seven sensors are divided over two arrays, which are both present in duplicate. This total of four arrays is also present as reference of the VOC's in ambient air. Patients will perform 5 tidal breaths, then, after a single deep inspiratory vital capacity maneuver and a 5 second breath hold, the patient exhales a vital capacity volume into the measurement setup. The exhaled air is directly measured by the SpiroNose, which is connected to an Ethernet cable for immediate secure data transmission to the online server. This technique has been applied to children older than 6 years without issues. The obtained breathprints will be securely stored in an online server; the BreathBase. Data can be downloaded for further processing and analysis with offline pattern recognition software.

The collection of exhaled breath for the GCMS analysis is very simple and does not bring any kind of burden to the child. The child will be asked to breath tidally through a mouthpiece into a breath sampling system, as described previously (77). Children breathe tidally through a

facemask connected to a non-rebreathing valve system, while watching cartoons. On the expiratory port of the valve, a 3-L polycarbonate bag (Tedlar® bag; SKC Ltd, Blandford Forum, UK) is connected to collect exhaled breath. After the bag is filled, it will be emptied across a stainless steel, two-bed sorption tube, filled with Carbograph 1 TD/Carbopack™ X (Markes International, Llantrisant, UK) for rapid adsorption and stabilisation of volatile compounds. The tubes are airtight capped and stored at room temperature until analysis. VOCs will be released from the tube using thermal desorption (Unity desorption unit; Markes International). Next, the sample will be injected into the gas chromatography (GC) capillary (Trace GC; ThermoFischer Scientific, Austin, TX, USA). In the GC capillary, VOCs are separated and subsequently detected and identified using time-of-flight (TOF) mass spectrometry (MS) (Tempus Plus; ThermoFischer Scientific). The remaining part of the breath will be used for other VOC sensing techniques like the SIFT-MS and the CNT chip sensor. The duration of the breath collection procedure is approximately 5-10 minutes. In case of resistance of the child, the measurements will be terminated. The collection and analysis of VOCs in the exhaled breath follows an standard SOP created and validated in our research group. (appendix III)

Universal Sniff Test (U- Sniff) odour identification test (6 – 17 years)

Olfactory function of all children ≥ 6 years will be assessed by the U-Sniff, an international odour identification test for children. This valid and reliable tool is able to distinguish children with normal smell function from those with a reduced sense of smell (84-86). Normative values from a large group of healthy children are available (86). The odour identification test includes 12 odours (apple, banana, butter, coffee, cut grass, fish, flower, lemon, onion, orange, peach, and strawberry), presented in pen-like odour dispensing devices (Sniffin' Sticks), which are positioned 2 cm in front of the patient's nostrils for approximately 3 seconds. Children are asked to identify each odour with the help of four descriptors (pictures and words). This test can be performed within 5 minutes and is generally liked by children

Procedure of blood withdrawal:

Blood samples for this study will be collected during the study visit for COPP-2 for immunological, nephrological and, if indicated, cardiac evaluation. If possible, the collection of the blood sample will be combined with a blood sample collection for routine clinical care. In this case, there will be no additional burden to the patient.

If no blood sample collection is scheduled for routine clinical care, a venapuncture will be done, giving some burden to the patient. To ensure that this burden is minimal, we will apply a topical anaesthetic (lidocaine/prilocaine or lidocaine/tetracaine as per local guidelines) prior to the procedure. Also, we will instruct local researchers to engage in "positive language" before and during the procedure. (87, appendix I) The amount of blood withdrawn depends on the body weight of the patient. With these amounts, we remain well below the recommended maximum of 3% of total blood volume in all weight categories (88). If the local investigator does not succeed in obtaining the required amount of blood, a smaller sample will be collected. In that case, blood investigations will need to be prioritized.

body weight	EDTA (mL)	serum (mL)	

3-5 kg 20-40 kg	4 20	1 10	
5-10 kg	5	2	
>/= 40 kg	30	20	
10-20 kg	10	5	

Immunological evaluation:

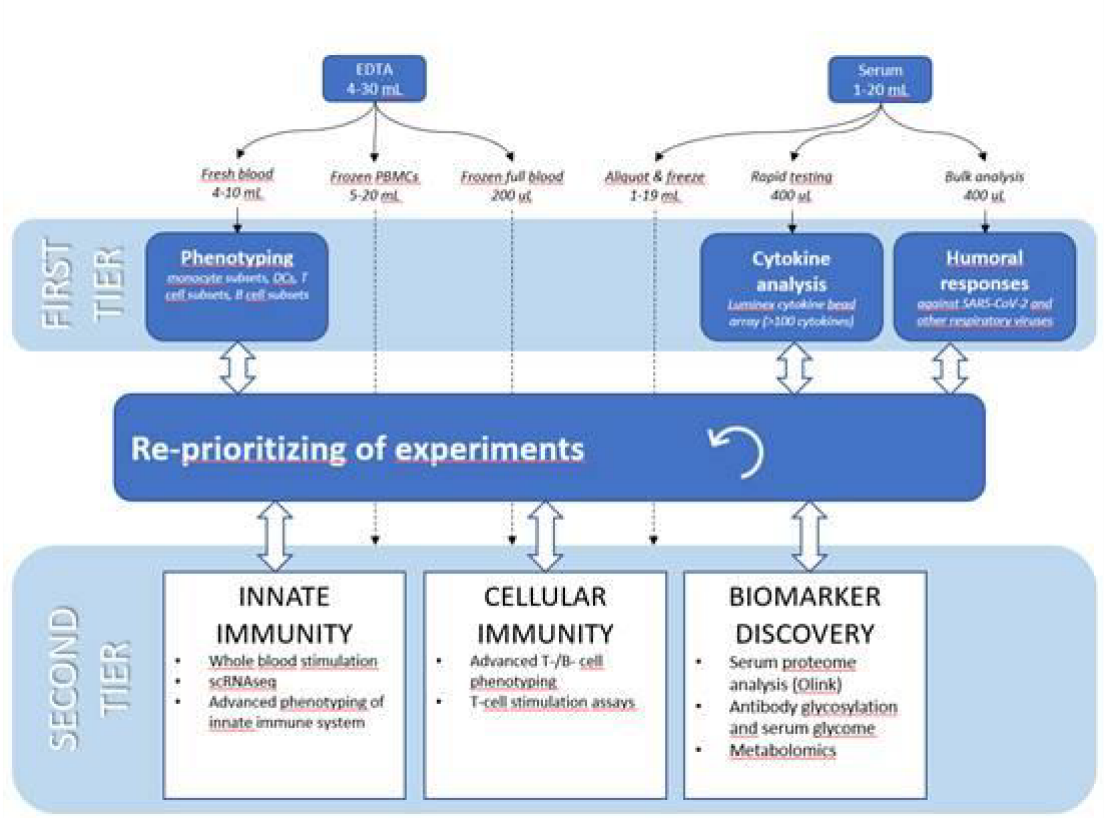
For this study, we will obtain a detailed immunological profile of children 4-12 months after COVID-19. Since we will have a limited amount of material available from each patient, we will do this in a two-tiered approach. This will enable us to choose what will be the most informative in-depth immunological analysis following a more general screening of the innate and adaptive immune system.

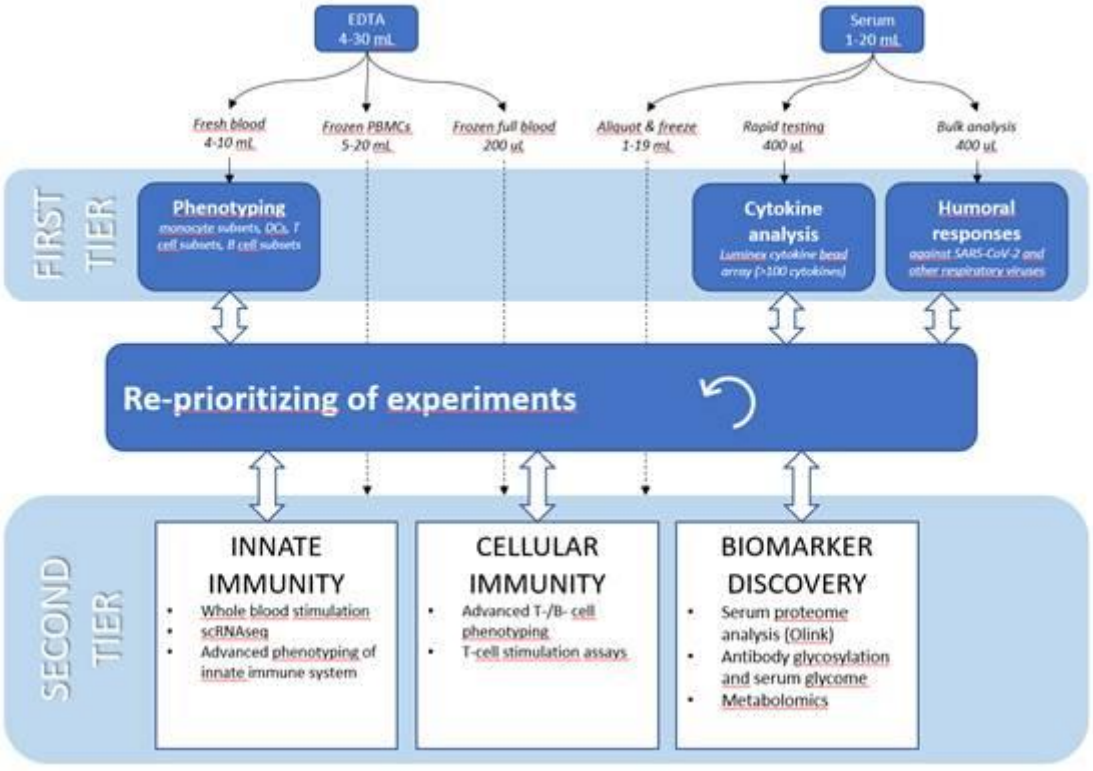
In the FIRST TIER we will perform a general assessment of the immune system. In this general assessment we will perform the following investigations:

- Detailed phenotypical analysis of the cells of the innate and adaptive immune system using multicolor flow cytometry. We will use the EuroFlow protocol PID Orientation tube to be able to identify and quantify the main leukocyte and lymphocyte subsets, using 12 markers: CD27, CD45RA, CD8, IgD, CD16, CD56, CD4, IgM, CD19, CD3, CD45, and TCRγδ. This will enable us to identify B-cells (including B-cell subsets), T-cells (including T-cell subsets), NK-cells, monocytes (including non-classical CD16+ monocytes), dendritic cells, basophils, neutrophils and eosinophils.
- Multiplex analysis of inflammatory cytokines using Luminex bead arrays (in excess of 100 analytes, to determine Th1/Th2 and innate responses).
- Quantitative and qualitative analysis of anti-SARS-CoV-2 antibodies and potential cross-reactive antibodies to other coronaviruses.

Depending on the results of the investigations in the first tier, we will choose one of the following in-depth immunological analyses for the SECOND TIER:

- The innate immune system (whole blood stimulation assays with cytokine production, single cell RNAseq, advanced flow cytometric phenotyping of the innate immune system).
- The adaptive immune system; with either a focus on T-cell responses (T-cell stimulation assays to evaluate effector responses against SARS-CoV-2 peptides), or B-cell responses (in-depth B cell subset analysis).
- Biomarker discovery of serum proteins using multiplex assays such as Olink or mass spectrometry


EDTA
4-30 mL
Serum
1-20 mL
Fresh blood
4-10 mL
Frozen PBMCs
5-20 mL
Frozen full blood
200 µL
Aliquot & freeze
1-19 mL
Rapid testing
400 µL
Bulk analysis
400 µL
FIRST TIER
Phenotyping
monocyte subsets, DCs, T cell subsets, B cell subsets
Cytokine analysis
Luminex cytokine bead array (>100 cytokines)
Humoral responses
against SARS-CoV-2 and other respiratory viruses
Re-prioritizing of experiments
SECOND TIER
INNATE IMMUNITY
Whole blood stimulation
scRNAseq
Advanced phenotyping of innate immune system
CELLULAR IMMUNITY
Advanced T-/B- cell phenotyping
T-cell stimulation assays
BIOMARKER DISCOVERY
Serum proteome analysis (Olink)
Antibody glycosylation and serum glycome
Metabolomics


EDTA
4-30 mL
Serum
1-20 mL
Fresh blood
4-10 mL
Frozen PBMCs
5-20 mL
Frozen full blood
200 µL
Aliquot & freeze
1-19 mL
Rapid testing
400 µL
Bulk analysis
400 µL
FIRST TIER
Phenotyping
monocyte subsets, DCs, T cell subsets, B cell subsets
Cytokine analysis
Luminex cytokine bead array (>100 cytokines)
Humoral responses
against SARS-CoV-2 and other respiratory viruses
Re-prioritizing of experiments
SECOND TIER
INNATE IMMUNITY
Whole blood stimulation
scRNAseq
Advanced phenotyping of innate immune system
CELLULAR IMMUNITY
Advanced T-/B- cell phenotyping
T-cell stimulation assays
BIOMARKER DISCOVERY
Serum proteome analysis (Olink)
Antibody glycosylation and serum glycome
Metabolomics

Retrieval of medical data from patient files:

Data regarding standard of care evaluation from patients included at COPP2 will be retrieved from the hospital files, after signed consent from parents/guardians and/or patients. This will include cardiac evaluation (cardiac ultrasound, ECG, cardiac MRI, cardiac markers), hematologic evaluation for post thrombotic sequelae (compression ultrasound and trombotic markers) and nephrological evaluation (nephrological markers in blood and urine) and neuropsychological follow-up.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1. Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Subjects will not be replaced after withdrawal

8.6 Follow-up of subjects withdrawn from treatment.

Not applicable

8.7 Premature termination of the study

Not applicable

9 SAFETY REPORTING

9.4 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The investigator will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.5 AEs, SAEs and SUSARs

9.2.1.Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. Adverse events are not expected to occur in the COPP2 study because it is a low risk observational study and therefore, there will be no need for adverse events to be reported.

9.2.2. Annual safety report

Not applicable

9.6 Follow-up of adverse events

Not applicable

9.7 Data safety monitoring board (DSMB)/safety committee

Not applicable

10 STATISTICAL ANALYSIS

IBM SPSS (SPSS Statistics for Window: IBM, Armonk, NY) or R Statistics software will be used according to applicable analysis.

10.4 Primary study parameters

Numerical data will be summarized using descriptive statistics: mean and standard deviations for normally distributed data, median and quartiles for non-normally distributed data. Group comparisons of independent samples will be done using the Student's t-test for normally distributed data and the Mann-Whitney-U test for non-normally distributed data. Dependent data will be compared using the paired student t-test and the Wilcoxon signed rank matched pair analysis.

Analysis from each research question described at section 8.1.1 and 8.1.2 will be conducted after the data analysis plan (DAP) to be formulated in consultation with the respective experts and experts in statistics.

10.5 Secondary study parameters

Secondary endpoints will also be summarized using descriptive statistics as mentioned above. Lung function indices, quality of life scores, and exercise capacity will be compared with standard reference sets obtained from normal controls.

1. Statistical analysis of immunological parameters: Relative distributions of immune cell subsets will be obtained by multicolour flow cytometry. Cytosplore or Infinicyt will be used for clustering and dimensionality reduction. Data will be imported in R for statistical analysis.
2. Cytokine levels will be analyzed in R and SPSS. Levels will likely be non-normally distributed in which case non-parametric Kruskal-Wallis tests will be done for comparison across groups. To adjust for multiple testing, we will use the two-stage step-up method of Benjamini et al.
3. Levels of neutralizing antibodies will be compared across subgroups using ANOVA in SPSS. Adjusted relative risks for specific outcome measures in association with immunological profiles will be calculated using a log-binomial model.

10.6 Other study parameters

Not applicable

10.7 Interim analysis

If abnormalities in exercise testing, attributable to COVID-19, is seen in less than 5% of 40 included patients performing exercise testing of which at least 20 with moderate to very severe COVID-19 (need of oxygen), we will discontinue exercise testing in children without cardiac or respiratory complaints.

11 ETHICAL CONSIDERATIONS

11.4 Regulation statement

This prospective cohort study is subject to the Medical Research Involving Human Subjects Act (WMO). This study will be conducted according to the principles of the Declaration of Helsinki (version 2013, 19-10-2013, (www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO), the General Data Protection Regulation (AVG), the Dutch Act on implementation of the General Data Protection Regulation (uitvoeringswet AVG) and other guidelines, regulations and acts. We will comply with the principles enshrined in the Council of Europe Convention on human rights and biomedicine – known as the Bioethics Convention Oviedo). Its main purpose is to protect individuals against exploitation.

11.5 Recruitment and consent

The recruitment and informed consent procedures will be done by researchers of the COPP2 study. Parents and/or children were asked consent during their participation in the COPP study to be approached for a follow-up study. The parents and children will be informed by email about this study by the investigators of the COPP study. This email refers participants to a separate website where they can enter their name, email-address, address and telephone number, so the researchers of COPP2 can call the participants and schedule the study visit.

In the event that the participation rate is less than 70%, the COPP investigators contact the parents and children directly, through the local investigator in all hospitals participating in the COPP study (23). They will receive their subject identification number from the COPP study, which they will give to the investigators of the COPP2 study, to ensure matching of the data from the COPP study and COPP 2 study. The investigators will provide the patient and parents with the appropriate patient information forms. They will be given at least 1 week to consider their decision and are free to reconsider their decision at any moment during the study. A research assistant will contact the participants and parents who are willing to participate, to schedule a study visit. Before start of the study visit written consent is obtained from both parents/caregivers.

Due to the current COVID-19 pandemic and the related precaution rules, it is in most part of the hospitals no longer allowed for both parents/caregivers to be present at a medical appointment at the outpatient clinic. For this situation there will be an exceptional procedure for the signature of the patient informed consent (PIF). The parent/caregiver who will not be present at the study visit will sign the PIF at home and this signed version will be taken to the hospital at the occasion of the study visit. The study investigator will make it clear for both parents/caregivers that they can contact the study investigator per telephone or email to ask for clarification at any time before signing the PIF.

Informed consent for the usage of the data of the COPP study is explicitly asked for.

11.6 Objection by minors or incapacitated subjects (if applicable)

The research will be conducted according to the Code of Conduct for Medical Research by the Federation of Dutch Medical Scientific Societies (Federa) and the code of conduct relating to expressions of objection by minors participating in medical research by the Netherlands Association for paediatric research.https://english.ccmo.nl/investigators/legal-framework-for-medical-scientific-research/codes-of- conduct/code-of-conduct-for-medical-research

Code of conduct

1. Individual children respond differently to diagnostic and treatment procedures and to participation in medical research. Various factors help to determine the nature of the response: the way the child is prepared for what is going to happen, the parent-child relationship, the doctor-patient relationship, the child-friendliness of the environment in which the procedure takes place and so on. One child will not be unduly disturbed by having an injection (even if he or she winces or makes some other display of pain), while another will find the experience distressing. Although responses vary considerably from child to child, there is a general correlation between the degree of 'invasiveness' of a procedure and the strength of the response. In some cases, fear regarding participation or a particular procedure will prompt a child to object. Patient and understanding explanation and reassurance will generally be sufficient to enable the research or the procedure to proceed without problems. Where a newborn child or infant is concerned, it is much harder to ascertain whether objection has been expressed. As a general rule, however, it is reasonable to suggest that a child may be deemed to object if its behaviour clearly differs in nature or degree from that normally displayed by the child when confronted with situations not encountered in everyday life. In this context, situations not encountered in everyday life may be considered to include diagnostic or therapeutic procedures.

2. Before seeking consent for a child's participation in medical research, an investigator must fully inform the child's custodial parent(s) or guardian about what is proposed. Information should be provided orally and in writing. The nature of the procedures involved in the research should be discussed with the parents and their views sought on the child's likely response. The possibility of the child objecting to participation and the type of behaviour that should be regarded as an expression of objection should also be discussed. The investigator should also explain what is to happen in the event of the child objecting. The consent obtained from the parents should include agreement to the proposed procedure for dealing with expressions of objection by the child.

3 The consent statement signed by parents should stipulate that, if the child should object to participation in the research, consent for its further participation will be invalidated.

4 If prior to the research there is doubt as to whether a child should participate, consideration may be given to involving the patient in the research for an agreed pilot period.

5 While the research is in progress, the behaviour of the child should be continually assessed at the research location to determine whether the child's behaviour is within the bounds normally associated with the child when confronted with situations not encountered in everyday life. If a child's behaviour is not within these bounds, he or she should be deemed to have expressed an objection in the sense of the WMO.

6 The parents, the investigator(s) and possibly a behavioural scientist should be involved in assessment of a child subject's behaviour. Assessment of a child subject's behaviour should not be a one-off exercise, but should continue through all phases of the research.

7 The parents of a child subject should be able to withdraw their consent at any point during the research. If a child subject expresses an objection, the child's participation should be discontinued.

8 In all medical research involving child subjects, the burden associated with participation should be minimised; where non-therapeutic research is concerned, the law stipulates that it must be negligible. Medical studies often involve the combination of research procedures with diagnostic procedures necessary in connection with the subject's treatment. Where research involves an invasive procedure, such as a finger prick or venepuncture, this should if possible be combined with a procedure necessary for diagnostic or treatment purposes, such as blood sampling. If possible, a needle or line that has already been inserted should be utilised, so that the number of 'jabs' is kept to the minimum. The burden can also be reduced by the use of plasters with local anesthetic. The various steps to be taken with a view to minimising the burden should be detailed in the research protocol and in the information given to the parents and subjects.

9 The following should be noted in the research file or the medical (status) report, as appropriate:
a the outcome of any trial participation;
b the consent of the custodial parent(s) or guardian, including the procedure to be followed in the event of a possible expression of objection;
c an account of the subject's participation in the research, stating whether objection was expressed;
d an assessment as to whether the subject's behaviour constitutes objection, as referred to above;
e the names of the people responsible for assessing the subject's behaviour, as described above;
f an assessment as to whether the subject's behaviour in the course of the study constitutes objection;
g the steps taken to minimise the burden associated with participation.

10 The protocol for a medical research project in which minors are to be used as subjects should state that the NVK's code of conduct for dealing with subjects' expressions of objection in the course of the research will be adhered to.

11 This code of conduct will be evaluated in consultation with the research community two years after its initial publication and amended as necessary.

This code of conduct was approved by the Board of the Netherlands Association for Paediatric Medicine (NVK) on 21 May 2001 and published in NVK Newsletter no. 3, June 2001.

11.7 Benefits and risks assessment, group relatedness.

Subjects do not benefit personally from study participation, although possible long term sequelae can be detected early and proper treatment if necessary can be initiated. This non-therapeutic research with minors will have negligible risks and some burden. The burden is low.

The results from this study will benefit the target group, i.e. children with COVID-19 related disease and possible long term morbidity, leading to early detection and hopefully treatment.

Participants will be asked to visit the participating site hospital once or twice, depending on the clinical characteristics and symptoms.

The main burden of participating in this study, next to blood withdrawal, is visiting the hospital, undergoing a physical examination, smell and taste tests, pulmonary function testing, exercise testing, exhaled breath collection, and completing 5 questionnaires. The expected required time-investment of the children and their caregivers is approximately 2-4 hours, depending on the age and willingness of the child. In case a child is included in the neurocognitive sub-study of COPP2 the second study visit will take approximately 2 extra hours. In the case of incidental findings, patients will be referred to their treating physician. To reduce the burden of travelling, patients will travel to the nearest participating hospital.

All children and/or their caregivers will be asked for consent to withdraw blood for immunological evaluation. If they are not willing, they can still participate in other parts of this study. The amount of blood drawn depends on the body weight of the patient, and ranges from 4 to 50 ml. To ensure minimal burden, the collection of the blood sample will be combined with a blood sample collection for routine clinical care whenever possible. To ensure that the burden associated to blood collection is minimal, we will apply a topical anaesthetic (lidocaine/prilocaine or lidocaine/tetracaine as per local guidelines). The procedure will only be performed by trained researchers who will follow the study SOP and make use of positive language before and during the procedure. (87/appendix I)Blood withdrawal without application of topical anaesthetic has a disco score of 1.71. The DISCO score is developed to aid researchers and ethics committees to evaluate discomfort in children during research procedures and uses a 5 point Likert scale (89), from not burdensome (1) to extremely burdensome (5).The DISCO score of blood withdrawal with application of topical anesthetic and/or using positive language is not known, without both it is 1.72. (90)

All patients above the age of 4 years old, will be subjected to pulmonary function tests. Pulmonary function tests are standard care in the follow-up of pediatric pulmonology patients, and are used as a screening test for pulmonary morbidity. The pulmonary function test has a DISCO score of 1.46 (33).

The research protocol and PIF's have been commented by both the JAR (jongeren advies raad, AmsterdamUMC hospital) and the Dutch pediatric patient foundation, called "Stichting kind en ziekenhuis", which both approved the version 8 of the protocol and version 7 of PIF and considered the pulmonary function tests as not burdensome.

This study includes only minors. Since COVID-19 in children is less severe than in adults, the long-term effects cannot be deduced from future adult studies. In adults, severe sequelae of COVID-19 are seen. In the future, the results of this study may lead to a better understanding for the need of follow-up of pediatric COVID-19 patients.

The family doctor or the general pediatrician of the child will be informed about participation in this study. In the event that incidental findings are discovered, patients will be referred to their general pediatrician or pediatric pulmonologist for further follow-up and necessary treatment.

11.8 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor was granted dispensation by the METC for the statutory obligation to provide insurance for damage to research subjects, because participating in this study is without risks.

11.9 Incentives (if applicable)

Patients and parents will receive compensation in travel costs based on travelled kilometers or costs of public transport. Participants will receive a small present for participation.

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.4 Handling and storage of data and documents

Extracted data are or will be coded, used and stored by the research team of the COPP study and COPP-IMM study (Buddingh, Mooij, van Asmuth) and data of the COPP2 study will be added by the research team of the COPP2 study (Hashimoto, Brackel, Terheggen). Patient data will be coded with a study number. The key to the code will be safeguarded by the research team. Documentation belonging to the trial will be archived for 15 years in the “Investigator File”. Persons who will be involved in the research will be listed in the Signature List with the corresponding tasks.

Data will be collected in Castor EDC. This is an AmsterdamUMC approved data storage program. The majority of the data registered at the CASTOR database will be done as direct entry during the study visit, this will comprehend the demographic data, physical examination, general clinical information and pulmonary symptoms. The source information for the laboratory tests performed as normal care (hematological, cardiac and PICU evaluation), lung function, exercise test and chest CT will be available primarily at the EPD from the patient and afterwards entered at the castor study database. Data from the odour identification tests will be directly entered in the castor study database. Data for immunological assessment will be analyzed at LUMC and these results will be available at the castor database.

Questionnaires for assessments of quality of life and parental perception of cognitive and executive function, will be filled-in directly by the patient (parents/caregivers) at a website developed for the COPP2 study using the KLIK platform. The KLIK questionnaires will be stored on VPS (KVM) server managed by hosting company TransIP. Daily backups are made to a backup server using backup software Acronis. These backups are encrypted with a strong password. Biomedia (part of the KLIK expert team) is responsible for the storage and backup of the data. Biomedia is ISO and NEN certified.

Neurocognitive data gathered with the Emma Toolbox for Neurocognitive Functioning (Konigs & Oosterlaan, 2020) will only contain performance data that cannot be directly linked to participating individuals. Data derived from specific neurocognitive measurements on sensory and motor functioning, information processing, attention, learning & memory and executive functioning will be subjected to established pre-processing pipelines creating summary

measures of neurocognitive functioning. In addition, network analysis will be used to determine the organization of the 'neurocognitive network'. The inverse difference in z-scores will be used as a measure of connectivity between neurocognitive processes. Global network parameters will assess the organization of structural connectivity at the level of (i) integration (i.e. characteristic path length), (ii) clustering (i.e. transitivity, modularity), (iii) hierarchy (i.e. assortativity) and (iv) small-world organization (i.e. small-worldness).

The company Breathomix and possible associated partners will have access to the data related and necessary for the breath analysis (Spironose). This will be limited to age of the participant (in years) and gender. The data sent to Breathomix will be coded and will not include any personal information that could lead to the direct identification of study participants. Breathomix will keep the data for 15 years.

Name, e-mail address, address and phone number, collected when recruiting patients, will be stored in a separate KLIK database, which can be linked to the Castor COPP database using a separate link (unique KLIK-link) maintained by the LUMC COPP and COPP-IMM team. LUMC will not have access to this KLIK database. After the PIF has been signed, the LUMC will provide AmsterdamUMC with the necessary information to link this personal information with the COPP and COPP-IMM study data.

The analysis from immunological markers from the blood will be performed at LUMC. For this immunological sub-study, samples will be labelled with the COPP2 CRF number and date of sampling and send to Leiden University Medical Center. At the LUMC, samples are registered in 'Sample Navigator'. The LUMC COPP / COPP-IMM team (Buddingh, Mooij, Von Asmuth, Van Schouwenburg) only has access to the pseudonymized data of COPP/COPP-IMM and not to patient-identifiable data of COPP-2. Data from immunological analyses will be stored on a datasafe in the LUMC and accessible to the LUMC-COPP team. For integral analyses of clinical data and immunological analyses, pseudonymized data will be shared between the AUMC COPP-2 study team and the LUMC COPP study team by means of sharepoint.

For the radiological evaluation, pseudonymized imaging results will be send to two investigators, both in Erasmus MC and AmsterdamUMC, they will fill in their report in the COPP2 castor database. Results of exercise testing are also pseudonymized and sent aindependent investigator in Amsterdam UMC. Results will be filled in the COPP2 castor database. The local investigator of COPP2 is then able to review these results and – when abnormal – will discuss this with the treating physician.

12.5 Monitoring and Quality Assurance

For this study a monitoring plan has been made according to the good clinical practice (GCP) guidelines. (see appendix K6 monitoring plan). An independent monitor will perform regular monitoring starting after the inclusion of the first subject and subsequently twice a year during the trial. After the study is finished, the monitor will conduct a final monitoring. The monitor's tasks during the visits are described in detail in the monitoring plan. The specific monitor plan will be discussed in detail with the CRU (see appendix B2 AMC appendix bijlage 2.0 Aanvraag Centrale CRU monitoring COPP2 study).

12.6 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

12.7 Annual progress report

The sponsor/investigator will submit a summary of the progress of the study to the accredited METC once a year. Information will be provided on the data of inclusion of the first subject, number of subjects included and number of subjects that have completed the study, serious adverse events/serious adverse reactions, other problems, and amendments.

12.8 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report within the results of the study, including any publications/abstracts of the study, toe the accredited METC.

12.9 Public disclosure and publication policy

Upon closure of the study, data will be reported in a scientific journal. Data published in articles (scientific or non-scientific) will not be traceable to the actual patients.

13. STRUCTURED RISK ANALYSIS

Not applicable.

14. REFERENCES

1. Zhu, N et al. A Novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med, 2020: 382(8): p 727-733.
2. Guan, W.J., et al., Clinical characteristics of Coronavirus Disease 2019 in China. N Engl J Med, 2020.
3. Huang, C., et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet 2020. 395 (10223):p 497-506
4. Wang, Y.,et al., Unique epidemiological and clinical features or the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. Journal of Medical Virology. n/a (n/a).
5. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatrica 2020, doi:10.111/apa.15270.
6. Dong Y, Mo I, Hu Y et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in children. Pediatrics 2020. DOI 10.1542/pes.2020-0702.
7. Lu, X., et al. SARS-CoV-2 infection in Children. N Engl J Med 2020.
8. Tagarro A, Epalza C, Santos M et al. Screening and severity of coronavirus disease 2019 (COVID-19) in Children in Madrid, Spain. Jama Pediatr, published online april 8 2020. Doi: 10.1001/jamapediatrics.2020.1346.
9. CDC COVID-19 response team. Coronavirus disease in children, United States, February 12-april 2, 2020. MMWR, volume 69, 1-5.
10. Liguoro I. Pilotto C. Bonanni M et al. SARS-COV-2 infection in children and newborns: a systematic revew. Eur. J. Ped 2020, May 18. Epub ahead of print.
11. www.covidkids.nl
12. Swann et al. Clinical Characeteristics of children and young people admitted to hospital with covid-19 in United Kingdom: prospective multicentre observational cohort study. BMJ 2020:3249: 1-15.
13. Whittaker E, Bamford A, Kenny J et al. Clinical Characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. JAMA 2020, June 8th, epub ahead of print.
14. www.covidkids.nl/scientific-dashboard/
15. Shekerdemian LS, Mahmood NR, Wolfe KK et al. Characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. JAMA pediatrics 2020, may 2011. Epub ahead of print.
16. Greenhalgh T, Knight M. A'court C et all. Management of post-acute COVID-19 in primary care. BMJ 2020:370:m3026
17. Sheehy LM. Considerations for postacute rehabilitation for survivors of COVID-19. JMIR Public Health Suveill 2020:6. Doi:10.2196/19462 pmid 32369030
18. Carfi A. Bernabei R, Landi F. Gemelli against COVID-19 Post Acute Care study group. Persistent symptoms in patients after acute COVID-19. JAMA 2020: doi: 10.1001/jama 2020.12603 pmid 32644129.
19. Tenforde MW. Kim SS. Lindsel CJ et all. VY Network investigators CDC COVID-19 response team NY Network investigators. Symptoms duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network. Univeted States. March-Juni2 2020. MMWR Morb Mortal Wkly Rep 2020: 69.993-8. https:www.cdc.gov/mmwr/volumes/69/wr/mm6930e1.htm. doi:10.15585/mmwr/mm6930e1 pmid 32730238
20. Assaf G. Davis H, McCorkell et al. An analysis of the prolonged COVID-19 symptoms survey by patient-led research team. Patent led research 2020.
21. Ngaj JC, Ko FW Ng SS et al. The long-term impact of severe acute respiartory syndrome on pulmonary function, exercise capacity and health status. Respirology 2010:15-543-50.
22. Panariello et al. Front Psychiatry 2020; 11: 582345 How SARS-CoV-2 affect the central nervous system? A working hypothesis
23. Brackel CLH, Lap CR, Buddingh EP et al. Paediatric long COVID, an overlooked phemonenon? Submitted.
24. Buonsenso D, Munblit D, de Rose C, et al. Preliminary Evidence on Long COVID in children. medRxiv 2021; : 2021.01.23.21250375.
25. Office of National Statistics. Updated estimates of the prevalence of long COVID symptoms. 2021 https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandlifeexpectancies/adhocs/12788updatedestimatesoftheprevalenceoflongcovidsymptoms

26. J.F. Ludvigsson. Case report and systematic review suggest that children may experience similar long-term effects to adults after clinical COVID-19. Acta Paediatrica, epub ahead of print November 17th, 2020.
27. https://www.dailymail.co.uk/health/article-8900501/Its-not-just-adults-children-hit-long-term-Covid-too.html
28. https://blogs.bmj.com/bmj/2020/10/16/counting-long-covid-in-children/
29. Li AM, So HK, Chu W et al. Radiological and pulmonary function outcomes of children with SARS. Pediatric pulmonology 2004: 38; 427-433.
30. Li AM, Chan CHY, Chan DFY. Long-term sequelae of SARS in children. Paediatric respiratory reviews 2005: 5: 296-99.
31. Yu CCW, LI AM, So RCH et al. Longer term follow up of aerobic capacity in children affected by severe acute respiratory syndrome (SARS). Thorax 2006: 61; 240-6.
32. Cui X, Zhang T, Zheng J et al. Children with coronavirus disease 2019: a review of demographic, clinical, laboratory and imaging features in pediatric patients. J Med Virol 2020:1-10.
33. Liu D, Zhang W. Pan F et al. The pulmonary sequalae in discharged patients with COVID-19. A short term observational study. Pulm Inf Dis. 2020 May 24;21(1):125.
34. Bernheim A, Mei X, Huang M et al. Chest CT findings in coronavirus diseaes-19 (COVID-19): relationship to duration of infection. Radiology 2020. 59;685-691.
35. Xia W, Shao J, Guo Y et al. Clinical and CT features in pediatric patients with COVID-19 infection: different points from adults. Pediatr Pulmonol 2020, mar 5. Doi: 10.1002/ppul.24718.
36. World Health Organization (WHO). Coronavirus. Geneva, Switzerland: WHO; 2020. https://www.who.int/health-topics/coronavirus#tab=tab_3. Accessed December 14, 2020.
37. Centers for Disease Control and Prevention (CDC). Your health. Symptoms of coronavirus. Atlanta, GA, USA: CDC; 2020. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html. Accessed December 14, 2020.
38. Cooper KW, Brann DW, Farruggia MC, et al. COVID-19 and the Chemical Senses: Supporting Players Take Center Stage. Neuron. 2020 Jul; 107(2):219–233.
39. Parma V, Ohla K, Veldhuizen MG, et al. More than smell – COVID-19 is associated with severe impairment of smell, taste, and chemesthesis. Chem Senses. 2020 Sep;45(7):609-622.
40. Menni C, Valdes A, Freydin MB, et al. Realtime tracking of self-reported symptoms to predict potential COVID-19. Nat Med. 2020; 26(7):1037-1040.
41. Yan CH, Faraji F, Prajapati DP, Boone CE, DeConde AS. Association of chemosensory dysfunction and Covid-19 in patients presenting with influenza like symptoms. Int Forum Allergy Rhinol. 2020 Jul;10(7):806-813.
42. Lechien JR, Chiesa-Estomba CM, De Siati DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. Eur Arch Otorhinolaryngol. 2020 Aug; 277(8):2251-2261.
43. Whitcroft KL, Hummel T. Olfactory Dysfunction in COVID-19: Diagnosis and Management. JAMA. 2020 Jun 23;323(24):2512-2514.
44. Moein ST, Hashemian SMR, Mansourafshar B, et al. Smell dysfunction: a biomarker for COVID-19. Int Forum Allergy Rhinol. 2020 Aug;10(8):944-950.
45. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. JAMA Neurol. 2020;77(6):683-690.
46. Sayin P, Altinay M, Cinar AS, Ozdemir HM. Taste and Smell Impairment in Critically Ill Patients With COVID-19: An Intensive Care Unit Study. Ear Nose Throat J. 2020 Dec;145561320977464.
47. Mak PQ, Chung KS, Wong JS, Shek CC, Kwan MY. Anosmia and ageusia: not an uncommon presentation of COVID-19 infection in children and adolescents. Pediatr Infect Dis J. 2020 Aug. 39(8):e199–e200.
48. Qiu C, Cui C, Hautefort C, et al. Olfactory and Gustatory Dysfunction as an Early Identifier of COVID-19 in Adults and Children: An International Multicenter Study. Otolaryngol Head Neck Surg. 2020 Oct;163(4):714-721.
49. King JA, Whitten TA, Bakal JA, McAlister FA. Symptoms associated with a positive result for a swab for SARS-CoV-2 infection among children in Alberta. CMAJ. 2020 Nov;cmaj202065.
50. Concheiro-Guisan A, Fiel-Ozores A, Novoa-Carballal R, et al. Subtle olfactory dysfunction after SARS-CoV-2 virus infection in children. Int J Pediatr Otorhinolaryngol. 2020 Dec;140:110539.
51. Stavem K, Ghanima W, Olsen MK, Gilboe HM, Einvik G. Persistent symptoms 1.5–6 months after COVID-19 in non-hospitalised subjects: a population-based cohort study. Thorax. 2020 Dec; thoraxjnl-2020-216377.

52. Woo MS, Malsy J, Pöttgen J et al. Frequent neurocognitive deficits after recovery from mild COVID-19. BrainComm 2020:23:2(2):fcaa205.
53. Almeria M, Ceiudo JC, Sotoca J, Deus J, Krupinski J. Cognitive profile following COVID-19 infection. Clinicalpredictors leading to neuropsychological impairment. Brain Behav Imm Health 2020:9:100163
54. Heneka et al. Immediate and long-term consequences of COVID-19 infections for the development ofneurological disease. Alz Res Therapy 2020:12-69
55. Swann OV, Holden KA, Turtle L, Pollock L, Fairfield CJ, Drake TM, et al. Clinical characteristics of children and young people admitted to hospital with covid-19 in United Kingdom: prospective multicentre observational cohort study. BMJ. 2020;370:m3249.
56. Oualha M, Bendavid M, Berteloot L, Corsia A, Lesage F, Vedrenne M, et al. Severe and fatal forms of COVID-19 in children. Archives de Pédiatrie. 2020;27(5):235-8.
57. García-Salido A, Leoz-Gordillo I, Martínez de Azagra-Garde A, Nieto-Moro M, Iglesias-Bouzas MI, García-Teresa MÁ, et al. Children in Critical Care Due to Severe Acute Respiratory Syndrome Coronavirus 2 Infection:Experience in a Spanish Hospital. Pediatric Critical Care Medicine. 9000;Online First.
58. Pathak EB, Salemi JL, Sobers N, Menard J, Hambleton IR. COVID-19 in Children in the United States: IntensiveCare Admissions, Estimated Total Infected, and Projected Numbers of Severe Pediatric Cases in 2020. Journalof Public Health Management and Practice. 2020;26(4):325-33.
59. Consiglio CR, Cotugno N, Sardh F, Pou C, Amodio D, Rodriguez L, et al. The Immunology of MultisystemInflammatory Syndrome in Children with COVID-19. Cell.
60. Weisberg SP et al. Distinct antibody responses to SARS-CoV-2 in children and adults across the COVID-19clinical spectrum. Nature Immunology 2021, 22: 25-31
61. Wechsler D. (2018). WISC-V-NL: Wechsler Intelligence Scale for Children, Fifth edition. Nederlandsebewerking. Technische Handleiding. Amsterdam: Pearson.
62. SE Raiford, X Zhou, LW Drozdick, Using the WASI®–II with the WISC®–V, 2016. Wechsler Intelligence Scale forChildren-Fifth Edition Technical Manual
63. Zhou, X., & Raiford, S. E. (2011). Using the WASI–II with the WISC®–IV: Substituting WASI–II Subtest ScoresWhen Deriving WISC–IV Composite Scores. Technical Report 2011.
64. Hurks P, Hendriksen J, Dek J, Kooij A. Accuracy of short forms of the Dutch Wechsler preschool and primaryscale of intelligence. Assessment 2016, 23(2), 240-249.
65. Königs, M. & Oosterlaan, J. (2020). Emma Toolbox for neurocognitive testing. Amsterdam, The Netherlands:Emma Children's Hospital University Medical Centres.
66. Königs, M., Verhoog, E., & Oosterlaan, J (2021). Exploring the Neurocognome: Neurocognitive NetworkOrganization in Healthy Young Adults. Under review
67. Van Widenfelt, B. M., Goedhart, A. W., Treffers, P. D., & Goodman, R. (2003). Dutch version of the Strengthsand Difficulties Questionnaire (SDQ). European Child & Adolescent Psychiatry, 12(6), 281-289.
68. Swanson, J. M., Schuck, S., Porter, M. M., Carlson, C., Hartman, C. A., Sergeant, J. A. & Wigal, T. (2012). Categorical and dimensional definitions and evaluations of symptoms of ADHD: history of the SNAP and theSWAN rating scales. The International Journal of Educational and Psychological Assessment, 10(1), 51.
69. Muris, P., Meesters, C., & van den Berg, F. (2003). The strengths and difficulties questionnaire(SDQ). European Child & Adolescent Psychiatry, 12(1), 1-8.
70. Gillijns, P., & Verhoeven, L. (1992). Het CITO leerlingvolgsysteem: Met het oog op de praktijk [The CITO pupilmonitoring system: Focus on practice]. Pedagogische Studiën, 69, 291–296.
71. Janssen, J., Verhelst, N., Engelen, R., & Scheltens, F. (2010). Wetenschappelijke verantwoording van de toetsen LOVS rekenen-wiskunde voor groep 3 tot en met 8 [Scientific justification of the mathematics test forgrade 1 until grade 6]. Arnhem: Cito
72. Mols, A., & Kamphuis, F. (2010). Wetenschappelijke verantwoording van de toetsen Spelling niet-werkwoorden voor groep 7 en 8 [Scientific justification of the spelling test for grade 5 and 6]. Arnhem: Cito
73. Weekers, A., Groenen, I., Kleintjes, F., & Feenstra, H. (2011). Wetenschappelijke verantwoording papierentoetsen Begrijpend lezen voor groep 7 en 8 [Scientific justification of the reading comprehension test for grade 5 and 6]. Arnhem: Cito.
74. Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. Eur Respir J 2005: 26: 319-38.
75. Cooper DM, Weiler-Ravell D, Whipp BJ, Wasserman K. Aerobic parameters of exercise as a function of

bodysize during growth in children. J Appl Physiol Respir Environ Exerc Physiol 1984. 56 (3): 628-34.

76. Klaassen EM, van de Kant KD, Jobsis Q et al. Exhaled biomarkers and gene expression at preschool ageimprove asthma prediction at 6 years of age. Am J Respir Crit Care Med 2015: 191(2): 201-7.
77. Neerincx AH, Vijverberg SJ, Bos LD et al. Breathomics from exhaled volatile organic compounds in pediatricasthma. Pediatr PUlmonol 2017: 52 (12); 1616-1627
78. Van der Schee MP, Paff T, Brinkman P et al. Breathomics in lung disease. Chest 2015 Jan: 147(1): 224-231.
79. Fens N, de Nijs SB, Peters S et al. Exhaled air moleculair profiling in relation to inflammatory subtype andactivitiy in COPD. European Respiratory Journal 2011: 38(6): 1301-1309.
80. De Vries R, Dagelet YW, Spoor P et al. Clinical and inflammatory phenotyping by breathomics in chronicairway diseases irrespective of the diagnotic label. Eur Respir J 2018: 11: 51 (1).
81. Brinkman P, van de Pol MA, Gerritsen MG et al. Exhaled breath profiles in the monitoring of loss of controland clinical recovery in asthma. Clin Exp Allergy 2017; 47(9): 1159-69.
82. Van der Schee MP, Palmay R, Cowan JO, Taylor DR. Predicting steroid responsiveness in patients with asthmausing exhaled breath profiling. CLin Exp Allergy 2013: 43(11):127-25.
83. Burges Watson DL, Campbell M, Hopkins C, et al. Altered Smell and Taste: anosmia, parosmia and the impactof long Covid-19. MedRxiv. 2020.
84. Schriever VA, Agosin E, Altundag A, et al. Development of an International Odor Identification Test forChildren:The Universal Sniff Test. J Pediatr. 2018 Jul;198:265-272.
85. Zou L, Dworschak A, Alizadeh R, et al. “U-Sniff” - the international odor identification test for children: anextension of its normative database and study of global reliability. Rhinology. 2020 Oct;58(5):471-476.
86. Gellrich J, Sparing-Paschke LM, Thieme T, et al. Normative data for olfactory threshold and odoridentification in children and adolescents. Int J Pediatr Otorhinolaryngol. 2019 Aug;123:5-9
87. Folder helpend taalgebruik. www.kindenziekenhuis.nl
88. Peplow C, Assfalg R, Beyerlein A, Hasford J, Bonifacio E, Ziegler A-G. Blood draws up to 3% of blood volume inclinical trials are safe in children. Acta paediatrica (Oslo, Norway : 1992). 2019;108(5):940-4
89. Staphorst MS, Rimman R, Passchier J, et al. The development of the DISCO-RC for measuring children’sdiscomfort during research procedures. BMC pediatrics 2017: 17-199.
90. https://vragenlijst.kindenonderzoek.nl/resultaten/longfunctietest/