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Collaborative On
Fatigue Following Infection

AKERSHUS UNIVERSITETSSYKEHUS

Long-Term Effects of COVID-19 in Adolescents (LoTECA)

Statistical analyses plan

NCT 04686734

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Revised #1: 31th of January 2022 (addition of post-COVID-19 syndrome (WHO definition) as endpoint)

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1. DESIGN

Design overview

Long-term effects of COVID-19 in adolescents (LoTECA) is a prospective study of the development of chronic fatigue and other sequels 6 months after the acute infection with SARS-CoV-2. A total of approximately 405 adolescents and young adults (12-25 years) with SARS-CoV-2 positive test (cases) as well as approximately 111 individuals with SARS-CoV-2 negative test (controls) will be included and followed prospectively for 12 months (Figure 1). The design enables analyses of risk factors for fatigue development as well as cross-sectional analyses between patients with persistent fatigue and recovered patients. The investigational program conducted at baseline and follow-ups includes clinical assessment, an extensive questionnaire package, autonomic cardiovascular control measures, spirometry, cognitive functions and biobanking of blood, urine, stool and hair.

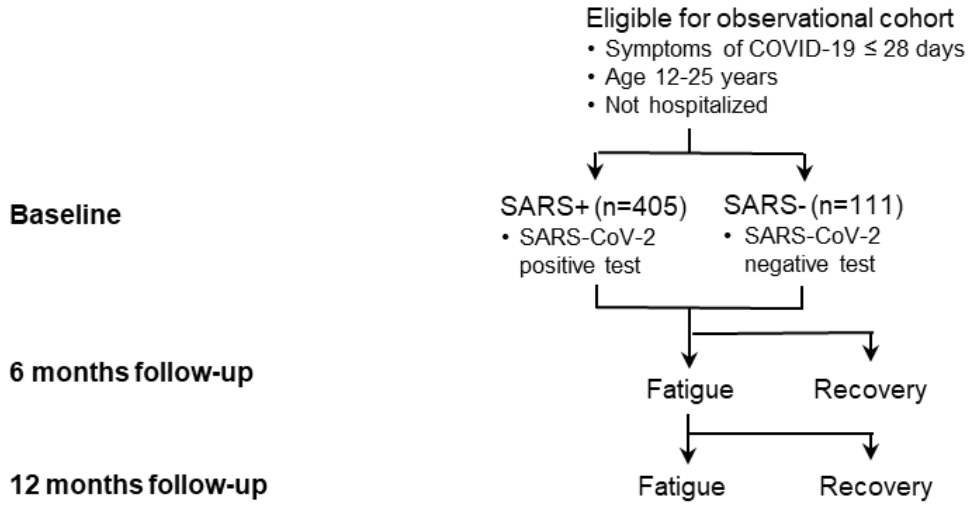

Figure 1. Design overview

Recruitment, inclusion and exclusion

The Microbiological Laboratory, Akershus University Hospital and Fürst Medical Laboratory are the main providers of microbiological analyses within the hospital's population area. Individuals with positive SARS-CoV-2 PCR-test as well as negative controls will be consecutively identified and invited to participate in the study through telephonic contact with the patient himself or one of the parents (depending on the age of the patient).

Table 1. Criteria for inclusion and exclusion

Inclusion criteria – cases Positive SARS-CoV-2 test Age 12-25 years ≤ 28 days since onset of first symptom	**Inclusion criteria – controls** Suspected SARS-CoV-2 infection Negative SARS-CoV-2 test Age 12-25 years ≤ 28 days since onset of first symptom
Exclusion criteria – cases Hospitalised because of COVID-19 Pregnancy Lack of consent from patient/next-of-kin	**Exclusion criteria – controls** Pregnancy Lack of consent from patient/next-of-kin

2. PRIMARY ENDPOINTS AND POWER CONSIDERATIONS

This study has two primary endpoints:

1. Chalder Fatigue Questionnaire total sum score at 6 months is the first primary endpoint (Chalder T, et al. J Psychosom Res 1993;37:147-53). A prospective risk factor assessment using baseline variables as potential predictors will be performed by linear regression modelling, A total of 500 included individuals yields a power of 0.9 to detect a variable that explains 2 % of the total variance (R^2) of fatigue score at 6 months (level of significance 0.05). Thus, the study is powered to detect even small effect sizes, which are considered important given the general lack of prior knowledge in the field.
2. Post-COVID-19 syndrome caseness at 6 months is the second primary endpoint (cf. https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.2). A case definition of post-COVID-19 syndrome was published by the World Health Organization in September 2021. Hence, this endpoint was added to the protocol during the course of the study, but before database lock. The appendix provides an operationalization of post-COVID-19 syndrome within the context of the present study. A prospective risk factor assessment will apply multiple regression modelling featuring binomial GLM analyses for post-COVID-19 caseness (eventually modified Poission-approach if the binomial model fails to converge).

3. VARIABLES

The primary endpoint is fatigue at 6 months (assessed by Chalder Fatigue Questionnaire total score). Relevant independent (explanatory) baseline variables include (but are not restricted to):

- *Background*: Sex, age, ethnicity, previous medical history
- *Initial clinical findings*: Symptom load/intensity, fever, routine blood haematology and biochemistry, etc.
- *Infectious load:* SARS-CoV-2 viral counts.
- *Immune function:* Increased general inflammation
- *Questionnaire results*: Emotionality (depression/anxiety, emotional awareness), personality factors (neuroticism, worrying), perceived loneliness, and negative life events.
- *Autonomic cardiovascular control*: Increased sympathetic nervous activity, decreased parasympathetic nervous activity
- *Cognitive functions*: Increased verbal memory, attention bias towards disease-related words, reduced working memory

4. ANALYSIS SETS

Full analysis set
The 'full analysis set' is defined as all participants who were subsequently included (n ~ 500). Missing values will be imputed based on the principle of 'multiple imputation' (MI).

Per protocol analysis set
The 'per protocol analysis set' is defined as all patients in the 'full analysis set' that completed the observation period (attended and completed consultation at baseline and at 6 months) without any of the following protocol deviations:

- Lost to follow-up
- Primary endpoint missing
- Diagnosed with COVID-19 during the follow-up period (controls only).

Missing data will not be imputed in the per protocol analysis set.

5. STATISTICAL METHODS

General considerations
Continuous variables will be reported with parametric (mean/standard deviation) or non-parametric (median, quartiles) descriptive statistics, depending on the distribution. Ordinal/nominal variables will be reported as frequency tabulation. All statistical tests will be carried out two-sided. A p-value ≤ 0.05 is considered statistically significant.

Prospective risk factor analysis
The relationship between each potential baseline risk factor variable primary endpoints are first explored in univariate linear regression analyses. As a second step, risk factors with a $p<0.2$ in bivariate analysis will be included in multiple linear regression modelling, in which p-values ≤ 0.05 will be regarded as statistically significant.

Cross-sectional analyses
At 6 months, fatigue cases will be defined as a dichotomous score of 4 or higher at the Chalder Fatigue Questionnaire. Patients thus defined as cases will be compared with recovered controls across a wide range of relevant pathophysiological variables, using parametric tests (Student t), non-parametric tests (Mann-Whitney) or table analyses (Chi-square, Fisher) as appropriate.

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Appendix: Operationalization of post-COVID-19 caseness (WHO definition) at 6 months

Variable	*Criterion*	*Comment*
Wording: "Post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include, but are not limited to, fatigue, shortness of breath, and cognitive dysfunction, and generally have an impact on everyday functioning. Symptoms might be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms might also fluctuate or relapse over time.»		
1. Persistent symptoms (cases must adhere to at least one)		
a) "… experienced altered smell and/or taste"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
b) "… experienced shortness of breath/dyspnea"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
c) "… experienced chest pain"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
d) "… experienced memory problems"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
e) Fatigue score	≥ 4	Chalder Fatigue Questionnaire, binomial scoring of single items. Definition of fatigue caseness.
f) PEM score	≥ 2 for at least 1 of 5 items	From the DePaul symptom questionnaire.
g) "….experienced palpitations"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
h) "… experienced concentration problems"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
i) "… experienced problems making decisions"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
j) "….experienced feeling of fever/chills"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
k) "… experienced cough"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
l) "… experienced dizziness"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
m) "… experienced headache"	≥ 3	Single item (5-point Likert scale, 3 indicates 1-2 times a week) from questionnaire
2. Functional disability (cases must adhere to the criterion)		
a) PedsQL (Pediatric Quality of Life); total score	≤ 80	Corresponds to a chronic disease of "mild" severity.

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3. Exclusion of other states that may explain persistent symptoms (screening followed by individual evaluation)

3.1. SCREENING (INDIVIDUALS MUST ADHERE TO ALL IN ORDER TO REMAIN AS CASES; NON-ADHERENTS ARE SUBJECTED TO INDIVIDUAL EVALUATION, CF POINT 3.2)

a) HADS-A (Hospital Anxiety and Depression Scale, anxiety subscore)	≤ 10	Screening for anxiety. Score of 8-10 corresponds to "possible" anxiety caseness, 11-15 corresponds to "probable" anxiety caseness[9]. A cut-off of 10 is reported to be optimal in a previous study of screening tools for psychiatric comorbidities in CFS/ME.
b) HADS-D (Hospital Anxiety and Depression Scale, depression subscore)	≤ 10	Score of 8-10 corresponds to "possible" depression caseness, 11-15 corresponds to "probable" depression caseness[9] A cut-off of 10 is reported to be optimal in a previous study of screening tools for psychiatric comorbidities in CFS/ME
c) KSQ (Karolinska Sleep Questionnaire, total score)	≥ 2	Screening for primary sleep disorder
d) NT-proBNP	Upper limit of normality (97.5 percentile)	Screening for cardiac failure. Upper limit (97.5-percentile) age 12-14 years is ≤242; 14-18 years is ≤207; above 18 year is ≤130 (women) and ≤86 (men).
e) SaO_2	<95%	Screening for respiratory failure
f) Other disorder/use of medications that may explain persistent symptoms	No one	As reported in questionnaire, eg. psychiatric, cardiac, pulmonary, or rheumatic disease.
g) Substance abuse that may explain persistent symptoms	No one	As reported in questionnaire
h) Finding during clinical examination that may explain persistent symptoms	No one	Eg. signs of cardiac failure
i) Finding from routine lab screening* that may explain persistent symptoms	No one	Eg. anemia

3.2. INDIVIDUAL EVALUATION OF POTENTIAL EXCLUSIONS (INDIVIDUALS **EXCLUDED** AS CASES MUST ADHERE TO ALL)**

a) Is the disorder/aberration causally related to the acute infection (COVID-19)?	No	Organ damage and/or psychological distress caused by the acute infection (COVID-19) itself is NOT a criteria for exclusion according to the WHO case definition (as opposed to the Fukuda-definition of PIFS).
b) Is it likely that the disorder/aberration is causally related to a persisting symptom?	Yes	Example: Chronic asthma may be causally related to persistent shortness of breath and/or coughs. However, chronic asthma cannot readily explain for instance problems of memory and concentration. If the latter problem persist, individuals may still be consider a case of post-COVID-19 syndrome.
c) Are there other persisting symptoms that cannot be explained from the disorder/aberration?	No	

*Routine lab screening included Blood Haemoglobin, Leukocytes, Differential count, Platelets; Plasma/Serum CRP, Vit B12, Folic acid, Ferritin, ALT, GT, LD, Albumin, CK, Glucose, HbA_{1C}, Bilirubin, D-dimer, INR, Urea, Creatinine, Natrium, Potassium, Calcium, TSH, Thyroxine

**Individual evaluation is performed independently by two researchers using all available information such as recorded data in the present project as well as patients' hospital and GP records. If disagreement about classification, cases are discussed with the principal investigator of the project until consensus is reached. A sensitivity analysis will be carried out excluding all cases with uncertain classification.

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Algorithm for caseness assessment