Alterations in microcirculatory oxygenation in long-COVID

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Primary Objective: To study alterations in microcirculatory oxygenation during exercise in patients with long-COVID in relation to clinical symptoms. Secondary Objectives: To study in patients with long-COVID compared to convalescent controls•…

Ethical review	Approved WMO
Status	Pending
Health condition type	Other condition
Study type	Observational invasive

Summary

ID

NL-OMON55916

Source
ToetsingOnline

Brief title
MICROX long-COVID

Condition

- Other condition

Synonym
post COVID syndrome

Health condition

long-COVID

Research involving
Human

Sponsors and support

Primary sponsor: Erasmus MC, Universitair Medisch Centrum Rotterdam
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: long-COVID, Microcirculation, Post-exertional malaise, Tissue oxygenation

Outcome measures

Primary outcome

I: Difference between groups (long-COVID versus control) in the change in microcirculatory Hb saturation (%) as determined by handheld sublingual reflective spectrophotometry before and after physical exercise (1 min sit to stand test).

II: Difference between groups (long-COVID versus control) in plasma levels of VEGF-A.

Secondary outcome

Disease characterization

Extensive disease characterization will be performed based on patient reported outcome measures (PROMs) collected online via Castor EDC:

1] Persisting symptoms and severity (corona symptom checklist)

2] Health-related quality of life (HRQoL, EQ5D)

3] Fatigue (Fatigue Assessment Scale, FAS)

4] Dyspnea (Modified Medical Research Council Dyspnea Scale, mMRC)

5] Cognitive failures in daily life (Cognitive failure questionnaire, CFQ)

6] Return to work (iMTA Productivity Cost Questionnaire, iPCQ)

7] Post-Exertional Malaise (modified sf-DSQ-PEM)

8] Postural orthostatic tachycardia syndrome (Malmo POTS symptom score)

9] Recovery status (Numeric scale and Likert scale)

Systemic oxygenation parameters (pulseoximetry) before and after exercise, difference between groups

• SpO2 (%)

Microcirculatory parameters (sublingual microcirculation): before and after exercise, difference between groups.

• Total vessel density (TVD in mm/mm2)

• Functional capillary density (FCD in mm/mm2)

• Perfused vessel density (PPV in %)

• Red blood cell velocity (RBCv in µm × s-)

Peripheral tissue oxygenation (near infrared reflectance spectrophotometry) before and after exercise, difference between groups

• Oxygen consumption derived by StO2 downslope (%/minute) during the ischemia phase of vascular occlusion test

• Microvascular recruitment determined as the StO2 upslope (%/minute) during the reperfusion phase of vascular occlusion test

Angiogenesis markers, difference between groups:

• Vascular transformation markers (HIF-1, ANGPT-1)

Endothelial activation markers, difference between groups:

• Serum markers of endothelial cell activation (E-selectin, VCAM-1, ICAM-1, P-selectin)

Inflammation parameters, difference between groups

• Leukocytes in the sublingual microcirculation (number of leukocytes per capillary-postcapillary venule unit per image sequence)

• Serum markers of inflammation activation (Il-6, sCD25 (sIL-2Ra), TNF*, IL-1*, Galectin-9, CXCL-10, sCD163, CCL2, CCL5, CCL7. CXCL9, IFN-*, IFN-*, C5a)

• Monocyte activation (apportioning to mature CD14+ CD16+ VEGF+ patrolling monocytes and higher monocyte pro-inflammatory gene expression)

• Plasma levels of neutrophil extracellular traps (NETs) (MPO-DNA complexes, extracellular DNA and citrullinated histone-3)

Coagulation parameters, difference between groups:

• Serum coagulation parameters (t-PA, PAI-1. PAI-2, vWF, FVIII)

• Myeloid-derived pro-angiogenic cells (PACs) types and subtypes

• The presence of microthrombi in plasma with confocal microscopy

• Circulating complexes of FXIIa-, FXIa-, kallikrein-C1q inhibitor, downstream thrombin-antithrombin complex

• Platelet activation (flow chamber, fluorescent platelet activation markers, P-selectin, GPIIb3a integrin, phosphatifylserine

• Thrombus formation potential (platelet adhesion, platelet activation, thrombus structure and fibrin clot formation) in flowing whole blood.

Mitochondrial function, difference between groups

- MitopO2

- MitoVO2

Study description

Background summary

The COVID 19 pandemic has had a profound impact, and the past years research has led to an accelerated effort towards understanding the consequences of SARS-CoV-2 infection. It is now known that COVID-19 is a multi-system disease affecting various organs in the body, characterized by a dysregulated immune response resulting in a cytokine release storm (1). Systemic inflammation as well as direct invasion of endothelial cells by the virus leads to endothelial dysfunction, which disrupt vasomotor function and can impair tissue perfusion and thus oxygenation (2) (3).

Microcirculatory tissue oxygenation
A substantial part of patients with COVID-19 disease develop long-COVID. This is defined as lasting symptoms, sequelae or abnormal clinical parameters existing twelve weeks or more after infection with SARS-CoV-2 onset (4). Symptoms include dyspnea, fatigue, and mental and cognitive disorders (5). A large group of patients are severely limited in physical exercise and report functional impairment, despite having normal cardiopulmonary function (6) (7), especially post-exertional malaise (PEM). PEM is the delayed onset of new symptoms or worsening of symptoms 12-48 hours after physical or cognitive exertion (8). Furthermore, as opposed to the systemic hypoxia in acute COVID19 infection, these patients have normal systemic oxygen saturation values (6) (7). Previous studies showed that symptoms of fatigue and subjective dyspnea were not related to impaired pulmonary gas exchange, with normal systemic pO2 and pCO2 during exercise exhaustion (9). However, peak VO2 was impaired in these patients during cardiopulmonary exercise testing, suggesting alterations in oxygen uptake and/or utilization in tissues. This was accompanied by decreased oxygen pulse, which is the ratio of oxygen consumption to heart rate and reflects maximal aerobic capacity. Given adequate systemic O2 levels, these data are pointing towards impaired oxygen regulation on the level of the microvasculature. Recent data support this by showing promising results of treatment with hyperbaric oxygen therapy and exercise with oxygen therapy (10) (11).

The microcirculation is a key player in regulation of oxygen delivery to tissue, and in healthy people recruitment of the microvasculature occurs in

response to metabolic demands such as during exercise. Interestingly, alterations found in the microvasculature of acute COVID-19 patients could reflect adaptations to the systemic hypoxia. A previous study from our group using direct observation and quantification of microcirculatory red and white blood cells sublingually using handheld vital microscopy (HVM), found increased capillary density and red blood cell availability, indicating recruitment of the microcirculation (12). Capillary hematocrit was increased, with a shift from red blood cells from the systemic- to the microcirculation. Possibly, capillary recruitment and recruitment of red blood cells to the microcirculation are adaptations to systemic hypoxia, in order to maintain tissue oxygen availability and extraction in the hypoxic state. Similar compensatory mechanisms are observed in experimental hypoxia (13) (14) but also in high-altitude studies (15) (16). These adaptations allow for toleration of low systemic arterial oxygen pressures. Interestingly, the most severely ill patients with COVID-19, as indicated by a SOFA score>10 points, did not show the increase in capillary density, nor the increase in capillary hematocrit. Possibly, the most severely ill patients lack this adaptive response to maintain tissue oxygenation.

In resting patients with long-COVID, a decreased density of small capillaries was found. This was accompanied by increased red blood cell velocity, possibly compensating for the loss of capillary density. However red blood cell velocity in the microcirculation was shown to be dependent of flow in feed vessels, which may suggest flow is dependent on these feed vessels (17). The flow being dependent on feed vessels could indicate impaired capillary recruitment to tissue demands. Another study showed that post-occlusion reactive hyperemia was altered after COVID-19 (18).

Mitochondrial dysfunction
A recent study demonstrated alterations in mitochondrial function in muscles from patients with long COVID who experience post-exertional malaise (PEM). This could imply that there are also defect on the level of O2 utilization, which contributes to tissue hypoxia and symptoms.

Angiogenesis
A consequence of tissue hypoxia on a microvascular level is induction of angiogenesis pathways. These encompass cellular responses triggered by cellular hypoxia, that eventually lead to the formation of new blood vessels. Vascular endothelial growth factor-A (VEGF-A) other angiogenesis markers angiopoeietin-1 (ANGPT1) and P-selectin were shown to be increased in patients with long COVID and predictive of development of long COVID syndrome (19). In line with this, the hypoxia inducible factor 1 (HIF-1) pathway was associated with COVID-19 that progressed to long-COVID which progressed proportionally with disease severity (20). So-called pro-angiogenic cells (PAC) (a subtype of monocytes) play important roles in the regulation of angiogenesis and neovascularization, primarily by the production of cytokines and other mediators (21).

Hypothesis and aims
These data suggest a key role for alterations in microvascular function and microvascular - not systemic - tissue oxygenation in the development of long-COVID syndrome. The endothelium has a vital role in maintaining microvascular function and allows the microcirculation to respond to tissue demands and increase flow when needed. Therefore we hypothesize that inflammatory-induced alterations in endothelial function lead to loss of normal anti-inflammatory and anti-thrombotic function of endothelial cells, which then contribute to impaired microcirculatory flow and oxygen delivery to tissues in long-COVID.

In this study we aim to unravel how symptoms of long-COVID patients are related to alterations in microcirculatory function and oxygen delivery, and the role of inflammation, endothelial activation and coagulation.

Endothelial activation
SARS-CoV-2 infection via ACE2 receptors leads to a severe inflammatory response, which helps to control and limit viral replication (1). However, the immune response can become dysregulated, leading to an excessive and prolonged inflammatory response, the "cytokine storm", which is the main contribution to tissue damage rather than direct infection with the virus. The cytokine storm involves the overproduction of pro-inflammatory cytokines, such as interleukin-1* (IL-1*), interleukin-6 (IL-6), interleukin-8 (IL-8). The pro-inflammatory cytokines cause endothelial activation with resulting loss of vasomotor function, but also of normal anti-inflammatory and antithrombotic function of endothelial cells.

Inflammation
IL-6 enhances the expression of adhesion molecules vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin on endothelial cells, which promote recruitment of leukocytes into the vascular wall (22) (23). Indeed, our group demonstrated there was an increased number of leukocyte aggregation visible in the microcirculatory vessels of COVID-19 patients (12). The level of leukocytes in the microcirculation was associated with higher disease severity (SOFA score). Previous studies have also shown that pro-inflammatory cytokine levels remain elevated after initial COVID-19 infection (24). In addition, the inflammatory response triggers formation of neutrophilic extracellular traps (NETs) which are formed upon neutrophil activation. They contain DNA and histones and form matrix networks of extracellular fibers that stimulate the cytokine storm further. Increased NETosis was demonstrated in patients with long-C

Study objective

Primary Objective:
To study alterations in microcirculatory oxygenation during exercise in patients with long-COVID in relation to clinical symptoms.

Secondary Objectives:
To study in patients with long-COVID compared to convalescent controls
• Extensive disease characterization (symptoms, quality of life, PEM)
• Systemic oxygenation at baseline and after exercise
• Microcirculatory tissue oxygenation and oxygen consumption at baseline and after
exercise
• Microcirculatory function and recruitment at baseline and after exercise
• Angiogenesis pathways (serum levels of angiogenesis markers)
• Endothelial activation (soluble markers of endothelial activation)
• Persistent inflammation (leukocytes, serum inflammatory markers, monocyte activation, NETs)
• Pro-coagulation (serum coagulation parameters, circulating coagulation complexes, platelet activation, thrombus formation potential, pro-angiogenic cells (PACs), the presence of microthrombi, vascular transformation markers of angiogenesis)
- In vivo and in vitro mitochondrial function

Study design

This study is a comparative, non-randomized, observational study. We will include a total of 54 long-COVID patients and 52 controls. For this study questionnaires will be taken online to characterize clinical symptoms in all patients. During a study visit, sublingual microcirculation and oxygenation measurements, as well as NIRS will be performed before and after exercise by a one-minute sit-to-stand test at the post-COVID outpatient department for 27 long-COVID patients and 26 controls. In addition, blood samples will be drawn to determine markers of angiogenesis, endothelial activation, inflammation and coagulation in all 54 long-COVID patients and all 52 controls.

The 27 long-COVID patients and 26 controls that underwent the entire protocol will be asked to return for a second study visit after six months. Then, the study protocol will be repeated and noninvasive in vivo mitochondrial function measurements will be performed.

Study burden and risks

The individual patients participating in this research will not benefit directly from the results of our research. Sublingual measurements of the microcirculation and tissue oxygenation are noninvasive and have no risk. They can however cause some discomfort, especially during the vascular occlusion test. Patients are asked to perform physical exercise with a one minute sit-to-stand test and this may cause fatigue. Blood withdrawal can cause discomfort. The noninvasive mitochondrial pO2 measurements are performed using a plaster which can cause skin irritation and pruritis, and hyperpigmentation when exposed to the sun.

The results could lead to new insights, that are highly relevant in understanding the pathophysiology of long COVID in general. The population of long COVID syndrome patients in the outpatient clinic is highly motivated to contribute to research aiming to unravel the pathophysiology of their disease.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Long-COVID patients
• Age >= 18 years, <65 years
• Past COVID-19 diagnosis, based on
o Positive PCR
o Positive Sars-Cov2 serology
o Positive rapid antigen test

o Typical clinical syndrome during the first pandemic wave, when testing was not possible
• Long-COVID-19 diagnosis based on World Health Organisation consensus diagnosis: (*Post COVID-19 condition occurs in individuals with a history of probable or confirmed SARS CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms and that last for at least 2 months and cannot be explained by an alternative diagnosis. Symptoms may be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms may also fluctuate or relapse over time)*
• Overall functioning <70% compared to functioning prior to onset of Long-COVID/ COVID-19 infection
• Long COVID duration > 6 months
• Presence of post-exertional malaise
• Provided written informed consent

Convalescent Controls
• Age >= 18 years, <65 years
• Past COVID-19 diagnosis, based on
o Positive PCR
o Positive Sars-Cov2 serology
o Positive rapid antigen test
o Typical clinical syndrome during the first pandemic wave, when testing was not possible
• No diagnosis of long-COVID, good recovery. Overall functioning >95% compared to functioning prior COVID-19 infection
• Self-reported general good wellbeing
• Provided written informed consent

Exclusion criteria

Long-COVID patients
• Unable or not willing to provide written informed consent
• Unable to complete written questionnaires in Dutch
• Unable to draw blood for study purposes
• Diagnosis of dementia
• Active treatment with hyperbaric oxygen treatment during study start
• Alternative diagnosis that may explain clinical symptoms
• Suffering from any pre-existing immune-driven disease or use of anti-inflammatory therapy of any kind (including NSAIDs and steroids) during the last 3 months
• Suffering from diabetes mellitus, hypertension, severe mental conditions or use of anticoagulant treatment in the past 4 weeks.
• No re-infection with COVID-19 in the past 3 months

Convalescent Controls

• Unable or not willing to provide written informed consent
• Unable to complete written questionnaires in Dutch
• Unable to draw blood for study purposes
• Diagnosis of dementia
• Genetically related to participating patients (e.g. brother/sister/parent)
• Suffering from any immune-driven disease or use of anti-inflammatory therapy of any kind (including NSAIDs and steroids), including during the last 3 months
• Suffering from diabetes mellitus, hypertension, severe mental conditions or use of anticoagulant treatment in the past 4 weeks.
• Re-infection with SARS-CoV-2 in the past 3 months.

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Pending
Start date (anticipated):	01-12-2023
Enrollment:	106
Type:	Anticipated

Medical products/devices used

Registration:	No

Ethics review

Approved WMO

Date:	30-11-2023

Application type:	First submission
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)
Approved WMO Date:	03-05-2024
Application type:	Amendment
Review commission:	METC Erasmus MC, Universitair Medisch Centrum Rotterdam (Rotterdam)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL85146.078.23