EFFECTS OF PHYSIOTHERAPEUTIC REHABILITATION ON CARDIOVASCULAR AUTONOMIC MODULATION AND INFLAMMATORY AND CARDIAC BIOMARKERS IN PATIENTS WITH LONG COVID

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OBJECTIVES

GENERAL OBJECTIVE

- To assess the cardiac autonomic function of patients with long COVID-19 before and after a physiotherapy rehabilitation protocol.

SPECIFIC OBJECTIVES

- To draw up a profile of patients with long COVID-19 treated in a rehabilitation programme.
- To investigate changes in cardiac biomarkers in patients with long COVID-19.
- Associating linear and non-linear HRV variables with clinical data (hospitalisation, number of symptoms, post-Covid time).
- Comparing the findings of autonomic modulation through linear and non- linear HRV variables at rest to inflammatory and cardiac biomarkers in different clinical groups of patients with Long COVID-19

METHODOLOGY

ETHICAL ASPECTS

This study will be carried out as part of a project approved by the Research Ethics Committee (CEP) of the State University of Pará (UEPA) under opinion number 4.288.736, which began in July 2020. This study will follow the precepts of the Declaration of Helsinki and the Nuremberg Code, respecting the rules of research involving human beings (CNS Res. 466/12 and 580/18) of the National Health Council. Participants will consent to the study by signing the Informed Consent Form (ICF).

TYPE AND PLACE OF STUDY

This is a controlled clinical trial on a local scale carried out in the cardiorespiratory diseases outpatient clinic at the Specialised Rehabilitation Centre (CERIII) in the Physiotherapy Teaching and Assistance Unit and

Occupational Therapy (UEAFTO) at the State University of Pará (UEPA) on the CCBS campus. This study will be registered on clinicaltrials.com.

SAMPLE CALCULATION AND SELECTION

The sample calculation will be carried out using the GraphPad StatMate application (SOFTWARE GRAPHPAD, SAN, CALIFORNIA), version 1.01, with significance of 5% and a test power of 99% to determine the sample N. The study sample will include patients who were assessed, underwent the full rehabilitation protocol and were re-evaluated. Data will be collected from 2024 to 2026. Participants with a clinical diagnosis of Long COVID-19, with an indication for physiotherapy rehabilitation, will undergo the cardiopulmonary rehabilitation protocol, which consists of morning or afternoon care, twice a week totalling 20 sessions.

INCLUSION AND EXCLUSION CRITERIA

Individuals with a confirmed diagnosis of COVID-19, of both sexes, aged between 20 and 59 years, recovered, in clinical condition for evaluation and undergoing clinical follow-up of Post COVID-19 at UEPA will be included. The criteria considered for the diagnosis of Long COVID-19 follow the criteria proposed by RAVEENDRAN, 2020: (a) acute symptomatic phase of COVID-19 confirmed by quantitative polymerase chain reaction (RT-qPCR) - reverse transcription, with symptoms consistent with COVID-19 not attributable to any other cause; and (b) at least one persistent COVID-19-related symptom, such as muscle weakness, chest pain, dyspnoea, fatigue, loss of balance, tremor, headache, myalgia, lower limb oedema, visual disturbances and/or insomnia, not attributable to another differential diagnosis, for at least four weeks after the onset of symptoms.

Exclusion criteria will be as follows: incomplete data, presence of a pacemaker, use of antihypertensive drugs that alter HRV, persistent pulmonary alterations, persistent desaturation, anaemia or presence of reinfection.

DATA COLLECTION

Prior to collecting data for the study, demographic and clinical information will be extracted from an interview with each individual by filling out an electronic form, including information such as: age, weight (kg), height (m), body mass index (BMI), previous illnesses, hospitalisation, length of stay, use of oxygen therapy, invasive mechanical ventilation, symptoms of COVID-19, HRV collection, among others. Together, this information will also be useful for including or excluding patients in the study (APPENDIX B).

HEART RATE VARIABILITY

The instruments used to measure HRV will be the Polar®RS800CX device with WearLink heart rate sensor, the elastic strap, the interface with USB input and a computer.

Participants will be instructed to abstain from caffeine, caffeine derivatives, heavy meals at least 24 hours before the test and strenuous physical activity. On the day of collection, the participant will rest for 15 minutes for haemodynamic stabilisation and then the HRV assessment will take place in accordance with the following steps: it will take place in the morning, in a quiet environment with a temperature between 22ºC and 24Cº, and heart rate will be recorded for 10 minutes in the supine position (lying down) using a Polar® RS800CX cardiofrequency meter (Kempele, Finland) on previously moistened skin using a strap/electrode below the xiphoid process. The patient will be instructed not to talk, move or sleep during the collection to avoid interfering with the signal.

In addition, vital signs will be collected (Heart Rate - HR; Respiratory Rate - RR; Blood Pressure - BP; Peripheral O2 Saturation - SpO2; Modified Borg Scale). A Lane Instruments® sphygmomanometer and a Bic® stethoscope will be used to collect systemic blood pressure (SBP). The Polar Pro Trainer 5 software (Polar Electro Ou, Finland) will then be used to capture the data.

For this analysis, the 5-minute section with the most stable signal will be selected, discarding the initial 30 seconds and the final 30 seconds of the collection. The linear variables analysed for HRV in the

of temporal frequencies in a specific routine according to Porta *et al*. (2001) will be the RR interval (iRR), Standard Deviation of all normal NN interval (SDNN), and Root-Mean of square successive NN interval difference (RMSSD), Percentage of Successive NN Intervals Differing by More Than 50 (PNN50), expressed in ms and the Stress Index (SI). In the frequency domain, the High Frequency (HF); Low Frequency (LF) and Ratio Between Low and High Frequency Components (LF/HF) will be analysed in normalised units (nu).

The non-linear variables observed were Standard Deviation of Instantaneous us Variability Beat to Beat Variability (SD1), Standard Deviation of the Long-term Continuous RR Intervals (SD2), expressed in ms.

LABORATORY TESTS

The patient will undergo the test after an 8-hour fast at UEPA's Clinical Analysis Laboratory, where the blood samples will be collected and analysed. 3 mL of blood will be collected in two Vacuette® tubes (Greiner Bio-One, Kremsmünster, Austria), one of them with anticoagulant ethylenediaminetetraacetic acid, for whole blood analysis and the other tube with separator gel and clot activator for serum analysis.

Cardiac function will be assessed by analysing creatine phosphokinase isoenzyme MB - CKMB (U/L), lactate dehydrogenase - LDH (U/L), C-reactive protein (CRP) and ferritin.

5.4.3 PROCEDURES

Stage 1: Approach eligible participants according to the inclusion criteria and interested parties after signing the informed consent form.

Stage 2: Screening of patients by interview to collect demographic and clinical data in order to identify persistent symptoms of COVID-Longa infection, even after the isolation period.

Stage 3: Participants will undergo laboratory tests and subsequent assessment of HRV and other tests required for physiotherapy rehabilitation.

Stage 4: The participants underwent the physiotherapy protocol, which consisted of aerobic training (treadmill or exercise bike) for 15 minutes, anaerobic training at the weight training station, performing exercises for the upper limbs (bench press and front pull) and lower limbs (180º leg press and extension chair) with weight determined by the 1 Repetition Maximum (1RM) test, performing 3 sets of 12-15 repetitions (depending on the session) and respiratory muscle training using POWERbreathe (30-40% of the MIP performed in the manovacuometry test).

Stage 5: The participants will be reassessed and the variables collected will be compared in the pre- and post-protocol periods. In addition, these values will be compared with the predicted values of the tests found in the literature.

STATISTICAL ANALYSIS

The collection data will be stored in Excel 2010™ software (Microsoft Corporation, Redmond, USA) and analyzed using Graphpad prism software version 5.0™ (Graphpad software, Inc., San Diego, USA). Student's t-test and ANOVA (analysis of variance) will be used to treat variables with normal distribution; Wilcoxon and McNemar will be used for variables that present an abnormal distribution, adopting a significance value of p<
0.05 p.

INFORMED CONSENT FORM (ICF)

You are being invited to take part in the research project entitled: ‘Effects of Physiotherapy Rehabilitation on Cardiovascular Autonomic Modulation and on Inflammatory and Cardiac Biomarkers in Patients with Long COVID’. The study aims to promote a therapeutic proposal through a functional rehabilitation programme for patients with manifestations of Long COVID-19.

I) The research will be carried out in room 17 at UEPA's Physiotherapy and Occupational Therapy Teaching and Assistance Unit (UEAFTO) on the CCBS campus.

II) You will be subjected to a specific treatment protocol comprising monitored functional training, with the aim of improving your tolerance to exertion, as well as receiving health guidance on the disease, its limitations, treatments and quality of life.

III) In this study, demographic and clinical data will be collected, as well as assessments. After selection, the 10-week treatment will take place twice a week, lasting an average of 50 minutes. Your vital signs (blood pressure, heart rate and respiratory rate) will be constantly checked.

IV) The expected benefits for you are related to a possible reduction in symptoms and greater tolerance to physical exertion, since specific treatment will be offered and you will be able to perform better in your activities of daily living.

V) The risks foreseen for you are related to intolerance to the proposed exercises, with alterations in monitoring signs such as heart rate, respiratory rate, blood pressure and peripheral oxygen saturation. If this occurs, the session will be interrupted until the signs and symptoms are fully restored and you will receive all the necessary support. Another risk you may face is the disclosure of your identity, but this risk will be minimised so that only the researchers have access to the information collected.

VI) You will not incur any expenses for taking part in this research, nor will there be any financial compensation for your participation. Any and all information obtained during both the assessment and the treatment will be kept confidential. The data will be stored and analysed together with that of other interviewees, and the identification of any participant will not be disclosed; your name will not appear in any publication, thus preserving your privacy.

VII) You can withdraw from the research at any time and this will not harm you in any way.

VIII) At any stage of this study, you will have access to the professionals responsible for the research to clarify any doubts you may have.

IX) This document is printed in two copies, one of which will remain with the researcher in charge and the other will be given to the participant. I would like to inform you that, at any time, you may seek clarification from the Researcher in Charge regarding the purpose of the research and the method and/or any other questions that may arise during the sessions.

( ) I wish to know the results of this research.

( ) I do not wish to know the results of this research.

Belém, _____of _____de _____

The Research Ethics Committee is responsible for the ethical monitoring of research involving human beings (Resolution 466/12) with the aim of guaranteeing the safety and well-being of research subjects.

I, _____, have discussed my decision to take part in this study with the researcher. It has been made clear to me what the purposes are, the procedures to be carried out and the guarantees of confidentiality and ongoing clarification. I agree to participate voluntarily in this study.

I declare that I have read the above information about the research, that I feel fully informed about its content, as well as its risks and benefits. I also declare that, of my own free will, I agree to take part in the research.

Telephone:_____ _____

Participant's signature

Researcher responsible: Luiz Fábio Magno Falcão

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