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Randomized, placebo-controlled study of corticosteroid therapy for persistent interstitial disease post-COVID-19.

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Abstract

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Introduction: The new coronavirus (SARS-CoV-2) is a virus with an intense capacity for dissemination and high mortality rate. The main cause of death is viral pneumonia, characterized as organizing pneumonia that responds to treatment with corticosteroids. After 1 month of the acute phase, 25% of patients have complete recovery from lung lesions. However, lung lesions can evolve as persistent interstitial lung disease; it is possible that this persistent disease is also responsive to corticosteroid therapy. There are no controlled and randomized studies on any treatment and its effect on the natural history of this subacute or late manifestation of COVID-19.

Objective: To understand the effect of oral corticosteroid therapy in the treatment of persistent pulmonary manifestations (clinical and radiological) in patients who had moderate, severe, and critical forms of COVID-19. To understand the role of some risk factors in the development of this form of lung disease. To add information on the natural history of interstitial lung disease secondary to SARS-CoV-2 pulmonary infection.

Methodology: Randomized, double-blind, placebo-controlled study of patients who had COVID-19 viral pneumonia. Patients included after 12 weeks of COVID-19 diagnosis with RT-PCR or imaging tests that confirm the infection for inclusion in the protocol 100 patients with changes in high-resolution chest tomography and diffusion spirometry, will be divided into two groups, placebo and treatment; the treatment group will receive prednisolone at a dose of 0.5 mg/kg/day for 1 month and weaning in 30 days. Clinical, laboratory, functional, and imaging evaluations will be performed at the beginning, after 3 and 6 months of treatment and monthly telephone calls.

The evaluation will include a medical evaluation aimed at the cardiopulmonary assessment, arterial blood gas analysis at rest and after 6MWT; functional assessment with spirometry with measurements of FEV1, FVC, TLC, and DLCO; functional assessment during exercise with 6MWT; functionality questionnaires with SF-36, MMRC, and PCFS; and collection of laboratory tests including inflammatory markers - D-dimers, blood count, C-reactive protein and ESR, autoimmunity markers (ANA and RF), and collection of medical and laboratory history data during the acute infection to verify correlation with residual lung disease. Imaging evaluation will be performed with high-resolution chest tomography.

Introduction

The first case of the new coronavirus (SARS-CoV-2) was recorded in Wuhan, China.

A virus with a high capacity for dissemination, after 4 months, there were already 200,000 deaths caused by COVID-19 worldwide, and high incidence has a high lethality rate.

Up to 20% of affected patients require hospitalization and oxygen therapy, more than 5% of those affected will require intensive care and orotracheal intubation or critical cases, and 2 to 3% will die regardless of the treatment instituted.

The coronavirus infects human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor, a receptor present in cells of the lung, heart, kidney, and intestinal tissue (WU et al., 2020).

In lung tissue, it causes damage to the alveolar epithelium, which directly impacts the reduction of the gas exchange area, leading to a decrease in the diffusion capacity of oxygen and carbon dioxide. After the acute phase of the injury, the repair phase of the damaged tissue begins, and the gas exchange area improves, in most cases, enough for the patient to be removed from mechanical ventilation, but there will be extensive lung injury that is still in the recovery phase. At the time, lung tissue injury with characteristics of DAD was already evident, with the presence of multinucleated pneumocytes, which are secondary to the aggression of these cells and proliferation of intrabronchial fibro granular tissue (similar to organizing pneumonia or bronchiolitis obliterans - BOOP) (TSE et al., 2004), evidencing lymphocytic alveolitis, acute fibrinoid injury, and organizing pneumonia (REMMELINK et al., 2020).

In fatal forms, there is DAD with deposition of fibrinous exudate, formation of hyaline membrane, hyperplasia and loss of type 2 pneumocytes, loss of continuity of the basement membrane, and thickening of the alveolar tissue (WIGÉN et al., 2020).

Some people are more susceptible to developing the severe form of coronavirus, such as men, the elderly, diabetics, and obese people. The justification for the worse evolution of the clinical picture for some groups to the detriment of others is that for

predisposed people, the severe form of coronavirus has a weaker innate and adaptive response to the action of the virus, which would explain why children and women are less susceptible to the severe pulmonary form (WIGÉN et al., 2020).

After acute lung injury, a process of repair of the alveolar structures begins with stem cells present in the tissue itself. Alveolar macrophages phagocytose the injured tissue, produce cytokines and growth factors involved in tissue repair, and stimulate the differentiation of these cells for the formation of new tissue and deposition of connective tissue. Injury to the pulmonary epithelium and endothelium occurs in the inflammatory phase of COVID-19, as there is dysregulation in the matrix of metalloproteinases (MO et al., 2020)(RAI; SHARMA; KUMAR, 2020). However, when extensive lung injury occurs and the deposition of excess connective tissue and migration of fibroblasts that become myofibroblasts forming fibrotic tissue (OJO et al., 2020). What remains unknown is why certain individuals develop sequelae due to the accumulation of myofibroblasts and excessive collagen deposition, while others recover quickly and completely (RAI; SHARMA; KUMAR, 2020).

The risk factors for the development of persistent interstitial lung disease are related to the greater severity of the disease and are older age, greater severity of lung involvement, increased lactic dehydrogenase (LDH) secondary to the extent of damage to lung tissue in the acute phase of the disease, stay in the intensive care unit, time of orotracheal intubation, smoking and alcoholism (OJO et al., 2020).

Another factor related to the severity of the condition is lymphopenia. The measurement taken at the beginning of the condition predicts a risk of greater lung damage and worse lung recovery since the reduction in lymphocytes causes a deficiency in viral clearance and greater damage to the organism (WIGÉN et al., 2020). The urea level at the beginning of the condition is also related to the aggressiveness of the kidney injury and predicts a worse lung prognosis.

The possible mechanism that would explain persistent lung injuries is that the virus when binding to angiotensin-converting enzyme 2 (ACE2) receptors, internalizes the receptor, and thus, the virus gains access to the cell and begins to attack the organism. Due to downregulation, there is a reduction in the expression of the ACE2 receptor. However, this receptor is part of the renin-angiotensin system, which plays

an important role in regulating the immune response and in the recovery of damaged tissue (WIGÉN et al., 2020). Thus, the process of regulating the inflammatory response and recovering lung tissue is impaired, which allows for greater aggression to the lung parenchyma.

There are some differences between the lung disease caused by the new coronavirus and other forms of acute respiratory distress syndrome (ARDS), despite the intense aggression, with lung involvement being the main predictor of severity and sequelae of all of them. COVID-19 has a peculiarity: it causes alveolar damage with low elastance, while others have high elastance. In addition, the computed tomography findings are different from the classic findings of ARDS due to other etiologies, and altered coagulation is also a finding exclusive to COVID-19. In addition to these typical characteristics, residual interstitial lung disease in COVID-19 is different from other fibrosing diseases, such as idiopathic pulmonary fibrosis and fibrosing interstitial lung diseases, as they are mainly marked by the involvement of the pulmonary endothelium, while COVID-19 is marked by the involvement of the alveolar epithelium (RAI; SHARMA; KUMAR, 2020).

Recovery from the pulmonary condition is a slow process, and patients remain symptomatic for a long period, with dyspnea being a very common symptom. Patients who recover, including those with mild symptoms, maintain fatigue in 53% of cases, dyspnea on exertion in 43%, and chest pain in 21.7%, showing that there is a functional pulmonary sequelae in patients who have recovered from COVID-19 (CARFÌ; BERNABEI; LANDI, 2020).

Functional tests to assess possible pulmonary sequelae show that even mild cases can have diffusion changes, and as the severity of the disease increases, the change becomes greater. Functional tests capable of detecting changes are the measurement of carbon monoxide diffusion (DLCO), with an average reduction of 47%, and total lung capacity, with a reduction of 25% after the end of symptoms. These data are even worse in more severe patients (MO et al., 2020), (NUSAIR, 2020). In a 3-month follow-up after hospital discharge, up to 25% of patients maintain diffusion changes (HUANG et al., 2021). Another follow-up study, after 3 months of hospital discharge, showed that up to 25% of patients maintain spirometric

changes. And, 9 out of 55 people evaluated (16.3%), even with mild symptoms of the disease in the acute phase, maintained diffusion changes (ZHAO et al., 2020).

Chest computed tomography is the exam that defines the severity of aggression against the lung parenchyma in a quantitative way. Extensive lesions, interstitial thickening, ground-glass opacities, irregular interface, thick reticular pattern, and parenchymal bands predict a greater degree of progression to residual interstitial lung disease.

A study that evaluated lung lesions in the short term after hospital discharge, with serial tomography scans, showed that there was an improvement in lung lesions in 4 weeks in up to 64.7% of patients (consolidations from 49% to 2%, focal ground-glass opacities from 17.7% to 9.8%, multiple ground-glass opacities from 80.4% to 23.5%, interlobular septal thickening from 80.4% to 35.3%, subpleural lines from 29.4% to 7.8%, irregular lines from 41.2% to 15.7%) and disappeared completely in 25.5% of patients (LIU et al., 2020). This shows that most of the changes are inflammatory and largely recover after one month of hospital discharge. However, persistent lesions can progress to persistent interstitial lung disease.

In another study, in patients who required mechanical ventilation, follow-up after 3 months showed complete recovery of lung lesions in only 2 of the 41 patients evaluated. In this study, 89% of patients still had ground-glass opacities according to computed tomography (CT), signs of reticulation and fibrotic bands, with and without parenchymal distortion, bronchiectasis and bronchiolectasis in up to 67% of cases, presence of emphysematous destruction or cavities, with worsening and of pre-existing emphysema. Some patients presented areas of air retention, but this was not a frequent finding, and traction bronchiectasis was rare. This study showed that pulmonary sequelae in patients who had extensive lung involvement are very common and can progress to permanent interstitial lung disease. Also, most people who required mechanical ventilation had changes in lung function and residual changes visualized on high-resolution CT (HRCT) (VAN GASSEL et al., 2021).

The evaluation after 6 months of hospital discharge of patients with COVID-19 showed persistent pulmonary changes on CT in 52% of individuals who did not require oxygen support and 54% of those who required mechanical ventilation,

noninvasive ventilation, or other ventilatory support. Regarding DLCO, even patients with the mild form of the disease may maintain alterations, present in 22% of patients who did not require oxygen therapy and in 56% of patients who required some type of ventilatory support (HUANG et al., 2021).

The treatment of the pulmonary condition still in the acute phase in patients requiring orotracheal intubation and oxygen therapy (DE BACKER; AZOULAY; VINCENT, 2020) demonstrated benefit with immunosuppressive doses of corticosteroid therapy due to a pattern of injury to the lung tissue with lymphocytic alveolitis, acute fibrinoid injury and organizing pneumonia (REMMELINK et al., 2020), all of which are acute alterations responsive to corticosteroid therapy.

In hospitalized patients, the use of corticosteroid therapy with dexamethasone and methylprednisolone has the potential to reduce mortality. Furthermore, the comparison between the two types of corticosteroids showed that the use of methylprednisolone was more effective (KO et al., 2021).

After the recovery period, interstitial lung disease persists in many patients, but the lesions are not characteristic of definitive fibrosis with honeycombing. According to RAI et al., 2020 (RAI; SHARMA; KUMAR, 2020), the studies reviewed in the literature designated the pulmonary sequelae as pulmonary fibrosis. However, in a recent study, it was called persistent interstitial lung involvement due to the lack of definitive characteristics of a fibrosing lesion and the presence of possible reversible lesions (WELLS; DEVARAJ; DESAI, 2021).

The evaluation time to confirm persistent lung disease and the period of natural recovery from the disease are still uncertain. However, data from the literature on the follow-up of severe pneumonia show that the radiological recovery time should be around six weeks. Regarding MERS and SARS-CoV-1, the radiological recovery time is around 12 weeks, with complete resolution of radiological alterations in two-thirds of patients during this period, and the maintenance of radiological alterations after this period indicates a high probability of permanent lung disease (GEORGE et al., 2020). This review also suggests a post-COVID-19 follow-up after 12 weeks with chest X-ray, diffusion measurements, and walk test to assess desaturation during exertion, and if any alteration is identified, a chest HRCT scan is

considered to assess interstitial lung disease. However, the initial assessment or screening performed only with a simple chest X-ray may lead to the non-identification of pulmonary sequelae due to its low sensitivity.

Follow-up of patients after 6 months of hospital discharge showed changes in chest X-rays in up to 25% of patients and reduced diffusion in 46%, but only 31% of patients had dyspnea on exertion with MMRC greater than or equal to 1 (FAVERIO et al., 2021), the most severe patients are those with greater impairment in diffusion capacity and changes in imaging tests (HUANG et al., 2021).

Studies on which treatment would be most effective for persistent interstitial disease have not yet been established in the medical literature, and the medications currently being tested are nintedanib, pirfenidone, chloroquine, and corticosteroids. Prophylactic measures such as protective mechanical ventilation techniques, measures to prevent pneumonia infections and other respiratory complications can reduce the risk of residual lung disease (RAI; SHARMA; KUMAR, 2020).

A study on the use of corticosteroids in patients with pulmonary sequelae showed significant improvement after the use of a dose of 0.5 mg/kg, but with rapid weaning and weaning beginning in the first week of use. This study did not have a control group, and patients were followed for 3 months only after inclusion and initiation of treatment (MYALL et al., 2021). The use of corticosteroids plays a crucial role in the treatment of inflammatory lung disease related to COVID-19, apparently in all phases of the disease.

Since COVID-19-related lung lesions in the acute phase are responsive to corticosteroid therapy, it is inferred that, for persistent interstitial lung disease that demonstrates an inflammatory pattern with alveolar involvement, corticosteroid therapy may also have an important role. There are no controlled and randomized studies on any treatment and its effect on the natural history of this complication of COVID-19.

Objective

To understand the effect of oral corticosteroid therapy in patients who had moderate to severe forms of coronavirus to treat persistent pulmonary manifestations with a radiological pattern of interstitial disease.

To understand the role of some risk factors for the development of pulmonary fibrosis in patients who had pulmonary infection by coronavirus with persistent interstitial disease.

To add information on the natural history of lung disease in patients who had moderate to severe forms of COVID-19.

Methodology

The study will be conducted at the Hospital das Clínicas of the Ribeirão Preto School of Medicine of the University of São Paulo. It will be a randomized, double-blind, placebo-controlled study of patients who had pneumonia caused by the novel coronavirus and were treated as outpatients, hospitalized, or who required oxygen therapy or orotracheal intubation.

Treatment: corticosteroid therapy with prednisolone at a dose of half a milligram per kilogram per day, lasting 1 month, which will be gradually withdrawn with a 50% reduction in the dose every 7 days, up to a minimum dose of 5 mg for 7 days before stopping completely. The placebo group will receive tablets identical to prednisolone 20 mg for 1 month, and then the reduction will be done with placebo tablets identical to the 20 mg and 5 mg tablets.

The identification of the bottles and the preparation of the placebo tablets will be carried out by a compounding pharmacy that will identify the labels by codes, and a researcher on the team will learn the content related to each code (placebo or prednisolone). This researcher will not have access to the study care or procedures.

Patients who received placebo took a similar number of tablets for similar time span.

Visits/Protocol: After 12 to 20 weeks of the diagnosis of pneumonia caused by the new coronavirus patients will be a standardized time. A medical evaluation, laboratory, imaging, and functional tests will be carried out with a view to immediate entry into the study. This will be considered the first visit. Patients will be randomized, and treatment will be given on the first visit. The evaluations will be carried out on this first visit (baseline) and will be repeated after 3 and 6 months from the first visit. The recruitment period will be 12 months, and the follow-up period for each patient will be 9 months. Depending on the study outcomes and the interest of the research team, this follow-up may be extended through a new research project.

In addition to quarterly assessments, these patients will be assisted with monthly telephone calls to check adherence and possible adverse effects.

If any patient is still using corticosteroid therapy, they will also be randomized and will be instructed to wean off the corticosteroid in use within 15 days and will receive the study treatment while reducing the dose of the previous corticosteroid until it is completely removed.

Randomization

Numbers 1 to 100 were randomly mixed up using a computer randomizer program. The first fifty were allocated as placebo and the last fifty as Treatment. Included patients received a number consecutively from 1 to 100. Only one researcher (EOV) knew to which group every number (patient) belonged, placebo or Treatment. This researcher labeled medicine vials accordingly.

Patients

One hundred patients will be included in the study, of which 50 will be in the control group and 50 in the treatment group.

Inclusion criteria:

● Over 18 years of age;

● Diagnosis of COVID-19 by RT-PCR (Reverse transcription polymerase chain reaction), a test that detects the presence of viral antigen in nasal and oropharyngeal secretions or chest tomography typical of COVID-19 according to radiological standardization for COVID-19 cases (Machnicki, 2021);

● Pulmonary involvement with persistent disease identified on high-resolution computed tomography of the chest;

● Reduction in DLCO (percentage of predicted), with measurements less than or equal to 80%.

Exclusion criteria

● Previous diagnosis of pulmonary embolism;

● Previous and decompensated heart failure with electrocardiographic changes, chest X-ray with increased cardiac silhouette, and signs in the clinical examination suggestive of heart failure;

● History of acute myocardial infarction in the last month or angina pectoris; Absolute contraindications to the use of corticosteroids or other comorbidities that prevent the use of corticosteroids such as uncontrolled diabetes, untreated glaucoma, current infections such as pneumonia or tuberculosis, and untreated psychiatric disorders;

● Pregnancy and lactation;

● Inability to perform stress tests such as walking tests and spirometry due to critically ill neuropathy or sequelae of stroke, among other limiting pathologies.

The patient will be informed of the risks related to corticosteroid treatment, and this information will be included in the consent form.

Structured Clinical Assessment

The initial, 3-month and 6-month assessments will include the following tests:

1 - ASSESSMENT OF LUNG FUNCTION

- Spirometry with CO (carbon monoxide) diffusion and lung volumes

The spirometry test will be performed in the pulmonology department using a carbon monoxide diffusion device. To perform the test, the patient will be instructed to sit upright, and a nose clip will be used. The technique used must follow standards of reproducibility, acceptability, and predictive value parameters for age, sex, and height, which will follow the guidelines of the American Thoracic Society/European Respiratory Society Statement/ERS 2005 (MILLER et al., 2005). Simple spirometry with the use of bronchodilator (pharmacodynamic test)

The simple spirometry test will be performed simultaneously with the diffusion test in the pulmonology department. The technique performed must follow reproducibility and acceptability standards, and the predictive value parameters for age, sex, and height will be followed according to the American Thoracic Society/European Respiratory Society Statement 2005 (MILLER et al., 2005).

2 - FUNCTIONAL CAPACITY TEST

- 6-Minute Walk Test

To assess functional capacity and check whether desaturation will occur during exertion, will be performed the 6-minute walk test at the Rehabilitation Center of the Hospital das Clínicas in a flat corridor measuring 30 meters in length. Patients will be instructed and encouraged to walk as fast as possible for 6 minutes, using standardized phrases every minute. Initially, they will be kept at rest to observe oxygen saturation values through pulse oximetry (SpO2), respiratory rate (RR), blood pressure (BP), heart rate (HR), and sensations of dyspnea (DS), and lower limb fatigue (LLF) using the Borg CR10 scale (BORG, 1990). BP measurements will

be taken with the individual in a sitting position and verified at rest immediately after the test and in the first, third, and sixth minutes of recovery. The 6MWT will be applied according to the guidelines of the American Thoracic Society (CRAPO et al., 2002). The expected distance traveled will be calculated using the equation developed for the Brazilian population (BRITTO et al., 2013).

3 - EVALUATION OF SUBJECTIVE FUNCTIONALITY

● SF-36 Quality of life questionnaire related to respiratory diseases:

This questionnaire assesses the quality of life in the last 4 weeks and includes the following assessment items: functional capacity, limitation due to physical aspects, pain, general health status, vitality, social aspects, limitations due to emotional aspects and mental health (CICONELLI et al., 1999) (appendix 1).

● Medical Research Council (MRC) dyspnea severity classification (KOVELIS et al., 2008), classification indicated to verify dyspnea related to physical efforts performed by the patient, classification related to symptoms of the last two weeks (appendix 2). Its score ranges from zero to four depending on the presence of dyspnea related to the intensity of physical effort. The Post-COVID-19 Functional Status Scale (PCFS) will be applied to assess patients' functionality at all hospital visits (KLOK et al., 2020) (appendix 3). This is a scale for assessing functional status based on the patient's self-report. It is a scale with classifications regarding muscle weakness, fatigue, depression, anxiety, shortness of breath, and memory loss. Its score ranges from zero to four, with a score of zero indicating no functional limitation and a score of four characterizing patients with severe functional limitations that require assistance for self-care.

All questionnaires will be applied by the doctor responsible for the project, will be applied during the clinical evaluation, and will be part of the patient's anamnesis; the data obtained will be included in the medical record.

4 - MEDICAL EVALUATION AND LABORATORY TESTS

The medical evaluation includes anamnesis, clinical examination (clinical pulmonary and cardiac examination and general clinical examination, including weight and height measurements), and measurement of vital signs (heart rate, respiratory rate, oxygen saturation by pulse oximetry, blood pressure). The medical evaluation aims to identify changes in pulmonary auscultation and signs of chronic respiratory disease, in addition to evaluating other pathologies that may be grounds for exclusion from the research project, such as heart failure, pulmonary embolism, recent heart attack, inability to perform the proposed tests, such as neuropathy in critically ill patients.

Laboratory tests will be included to check for possible persistent inflammatory activity of the coronavirus in the body since there is still inflammatory lung disease. These tests include inflammatory markers, which are measurements of D-dimers, blood count, C-reactive protein, LDH, and erythrocyte sedimentation rate (ESR).

Some lung alterations may be pre-existing and secondary to pre-existing autoimmune diseases. Autoimmunity markers will be performed for differential diagnosis of autoimmune lung diseases: antinuclear factor (ANA) and rheumatoid factor (RF). If it is confirmed that these patients have interstitial lung disease associated with autoimmunity and overlapping with a disease secondary to SARS-CoV-2, these patients will be analyzed separately at the end of data collection.

The requested tests are part of the evaluation of patients who were hospitalized with a diagnosis of COVID-19 pneumonia and are included in the post-discharge reassessment routine.

A review of medical records to collect medical history and data on severity characteristics during hospitalization is necessary to obtain correlation data between this information and interstitial lung disease. The following variables will be evaluated to verify this correlation:

● sex, age, body mass index, previous diagnosis or during hospitalization of diabetes, systemic arterial hypertension;

● survey of intubation time and length of stay in the ICU bed;

● The extent of pulmonary involvement seen on tomography or chest X-ray at admission;

● level of urea, LDH, and D-dimers at hospital admission;

● History of smoking;

● History of alcoholism;

● Symptoms persisting after hospital discharge secondary to COVID-19 infection: chronic cough, dyspnea on exertion (dyspnea classification by MRC - appendix 2), chest pain, chronic fatigue, and joint pain.

Follow-up telephone calls will be made monthly to increase adherence and check for possible adverse effects of corticosteroid use.

Ethical aspects

The project will be submitted for review by the Research Ethics Committee of the Hospital das Clínicas de Ribeirão Preto through the Plataforma Brasil. Information about the objectives, procedures, risks, and benefits of the study will be passed on to patients, and upon their agreement to participate, they will be invited to sign an informed consent form (ICF).

Statistical analysis

ANOVA was used to assess continuous variables (FEV1 liter, FEV1%, FVC liter, FVC%, TLC liter, TLC%, DLCO, MIP, MEP, 6MWT distance, ESR, CRP, D-dimer).

SF-36 scores were analyzed using a negative binomial regression model with repeated measures and a logarithmic link function, given that we can consider these outcomes as discrete and non-continuous quantitative variables. This model was used because the assumptions of normality and homoscedasticity of residues for the ANOVA analysis were not valid. From the model estimates, the relative increase (or

reduction) in the mean was calculated based on the expression AR(β) = [exp(β) – 1]*100%.

MRC dyspnea score and PCFS scale are ordinal categorical variables; the analysis used an ordinal multinomial regression mode, which estimates the odds ratio of higher "scores", meaning worse.

The analysis of the dichotomized PCFS used a log-binomial regression model, which estimates the prevalence ratio of grade 3 or 4 occurring between groups and times.

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