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Effect of immunonutrition on serum levels of C-Reactive Protein and lymphocytes in patients with COVID-19: Randomized controlled double-blind clinical trial

Rodrigo Fernandes Weyll Pimentel[1], Magno Conceição das Merces[1]

[1]Universidade do Estado da Bahia

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Rodrigo Fernandes Weyll Pimentel

ABSTRACT

Background: Patients contaminated with COVID-19 undergo changes in leukocyte count, respiratory disorders, and an increase in inflammatory substances. To improve the inflammatory condition, some nutrients can be used, including arginine, omega 3 fatty acids and nucleotides. This study aims to evaluate how oral immunonutrient supplements affects serum C-Reactive Protein (CRP) levels and lymphocyte count in patients with COVID-19.

Methods: In this double-blind clinical trial we randomized 43 adult patients with COVID-19 to receive a standard high protein normocaloric supplement (control) or an immunonutrient-enriched supplement (experiment) for 7 days. The primary outcome was to evaluate changes in total lymphocyte count and serum level of CRP. The assessment of risk and nutritional status of these patients was also performed.

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1TRIAL PROTOCOL

1.1Study design

This is a phase-IV, longitudinal, prospective, analytical, controlled, randomized, 1:1 and double-blind clinical trial.

Figure1. Diagram of the study design.

1.2 Location

The study was carried out at a hospital specialized in caring patients diagnosed with COVID-19 in the city of Salvador, Bahia. This hospital is located at Praça Conselheiro João Alfredo, s/n, Pau Miúdo.

Founded on October 28, 1982, this hospital initially had 153 beds distributed among urgency, emergency, orthopaedics, paediatrics, medical and surgical wards of several specialties, as well as an ICU. Additionally, he had bioimaging services with ultrasound, echocardiography, and Doppler appliances. The facility also served as a field of internships for several health professionals under education at technical and academic levels.

With the Covid-19 pandemic, the institution underwent changes and was adapted to care exclusively for patients affected by SARS-CoV-2. The hospital now has specialized beds distributed in 6 ICUs and 2 wards, which admitted patients referred by the public health network through the Regulation Centre of the state of Bahia.

1.3 Population

All patients (total of available beds) admitted to the ward with a diagnosis of COVID-19 were studied over a six-month period (from July to December 2020), who met the eligibility criteria. Patients were prospectively followed up to a maximum period of 8 days.

1.3.1*Eligibility*

Inclusion criteria

Adult patients aged between 18 and 65 years, diagnosed with COVID-19 through molecular examination (RT-PCR), with patent gastrointestinal tract, fed with oral diet, and not under mechanical ventilation or requiring hospitalization in ICU.

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Exclusion criteria

The following patients could not participate in the study: pregnant; patients submitted to artificial nutrition in the 15 days prior to inclusion in this study; patients allergic to any components of the diets used; individuals with severe hyperglycaemia (> 180mg/L) or hypertriglyceridemia (>400mg/L); patients with previous gastrointestinal diseases (surgical resections, malabsorption syndromes, inflammatory bowel diseases, persistent paralytic ileus, upper gastrointestinal bleeding or severe acute pancreatitis); patients with immunosuppression defined by neutropenia, myelodysplastic syndromes, congenital immunodeficiency or Acquired Immunodeficiency Syndrome (AIDS), submitted to immunosuppressive therapies and systemic chemotherapy in the last 3 months; patients submitted to autologous bone marrow transplant last year, halogen bone marrow transplant in the last 2 years, patients with Graft Versus Host Disease(GVHD); individuals with advanced chronic diseases (Child-Pugh stage C, grade IV heart failure, functional stage-IV chronic lung failure, terminal degenerative neurological processes, neoplasms in remission or progressing under treatment); individuals with processes of short life expectancy including end-stage chronic kidney disease; individuals with acute processes of short survival, such as shock of any aetiology with multiple organ dysfunction refractory to therapy in the first 48 hours, or individuals submitted to post-cardiopulmonary resuscitation with severe neurological damage within 72 hours.

1.4 Randomization of participants

After applying the inclusion and exclusion criteria, forty-three patients were randomized at the end to receive standard high-protein normocaloric supplement (control) or supplement enriched with immunonutrients (experiment) in a 1:1 ratio, for 7 days. The randomization was simple and performed through a simple, random drawing on an internet site called *Research Randomizer*(RESEARCH RANDOMIZER, [*nd*]).

1.5 Administration of study treatments

Supplements were blinded to patients and investigators.

A pilot study was carried out with a sample referring to 10% of the total population for aligning the research protocol. To analyse the evaluation error, the Kappa coefficient was used to assess the agreement between the evaluators. Kappa index was < 0.80 and was considered a good correlation(LANDIS; KOCH, 1977).

1.5.1 *Control group supplement*

Participants in the control group received 2 200ml units of a high-protein normocaloric nutritional supplement without the addition of any immunonutrition component (Nutren Senior®, Nestlé) distributed over 24 hours. Every 100ml this supplement provided 98kcal, 8.0g of protein, 9.4g of carbohydrate, 3.2g of fat, without fibre and lactose.

Figure2. Nutren Senior®, Nestlé

1.5.2 *Experiment group supplement*

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Participants in the experiment group received 2 200ml units of a normocaloric, high-protein nutritional supplement with some L-arginine, nucleotides, and ω-3 essential fatty acids (Impact®, Nestlé) distributed over 24 hours. Every 100ml this supplement provided 109kcal, 6.5g of protein, 14g of carbohydrate, 2.8g of fat, without fibre and lactose.

Figure3. Impact®, Nestlé

1.5.3 *Dispensing and administration form of supplements*

Over 7 days, the supplements were dispensed in 300ml disposable cups, covered and without any identification that referred to the product name. Supplement's delivery started on the day of participant's inclusion in the study, and it was organized by the hospital's clinical nutrition team through the maids who also did not know which product they were delivering.

When the patient was discharged before ending the supplement period, a researcher went to the patient's home to deliver the diets daily, checking their immediate consumption.

The drug treatment instituted to control the symptoms caused by SARS-CoV-2 infection followed the institutional protocol and it was equally prescribed for all patients.

1.6 Monitoring of participants

Participants were monitored by researchers for 8 days, involving risk assessment and nutritional status, collection of blood samples for haematological (complete blood count) and biochemical (CRP) exams, as well as recording possible clinical complications.

1.6.1 *Risk assessment and nutritional status*

Participants were evaluated by the researchers on the day of inclusion in the study (D0). Nutritional Risk Screening 2002 (NRS 2002) tool (NRS 2002) was used for assessing nutritional risk(KONDRUP, Jens *et al.*, 2003). This instrument is the method recommended by the European Society for Clinical Nutrition (ESPEN)(BARAZZONI *et al.*, 2020b; KONDRUP, J. *et al.*, 2003). In Brazil, it is also the most suitable method for tracking nutritional risk for hospitalized patients. To classify them as having high nutritional risk, a score ≥ 3 was considered. Those with a score < 3 were classified as low nutritional risk.

In addition, all participants were weighed and had their heights recorded, they were barefoot and wore light clothing. The individuals were positioned in the centre of a digital scale (Welmy-W300A®, São Paulo, Brazil), in upright position and had their weight distributed in both feet. Body weight was recorded in kg, with three digits (00.0 kg) immediately after reading. For height recording, the participant was positioned barefoot in a stadiometer, with the head free of accessories, erect, with arms extended along the body, head held high and looking at a fixed point at eye level. Height was recorded in meters by using three digits (0.00 meters).

1.6.2 *Blood sample collection*

Participants underwent a blood sample collection in a peripheral vein (basilic or cephalic one) on D0 and after the supplement use period on the 8th day (D8). These samples were identified and sent to the clinical analysis laboratory for total lymphocyte count and CRP measurement.

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1.6.3 *Clinical and laboratory analysis*

1.6.3.1Blood count

The EDTA venous blood samples were automatically processed, and blood count, platelets, and erythrocyte diameter distribution (DDE) were obtained by means of an electronic cell counter. From the haematological data obtained, the total lymphocyte count was used for this clinical trial.

1.6.3.2 CRP dosage

CRP was quantified based on monoclonal antibodies by using the immunoturbidimetry method (high sensitivity), which is considered the gold standard for detecting CRP levels in an automated equipment.

1.6.4 *Medical records*

During the research period, some data were retrieved from the records found in the participants' hospital records to complement the data collected by the researchers.

Figure 5 summarizes the intervention steps and monitoring of participants during the study.

Figure4. Diagram of Intervention and follow-up of the participants included in the study.

1.7 Outcomes evaluated

1.7.1 *Main outcome*

The main outcome of the study was to assess changes in total lymphocyte count and serum CRP level.

1.7.2 *Secondary outcomes*

The secondary outcomes of the study were related to patients' nutritional risk and nutritional status according to BMI.

1.8 Efficacy and therapeutic failure criteria

Efficacy criterion for oral supplement with immunonutrients was to promote an increase in the total lymphocyte count and a reduction in CRP levels greater than or equal to 30%. Therapeutic failure (i.e., the lack of the expected effect) was considered when the increase or decrease, respectively could not be observed at such levels.

1.9 Adverse events

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Serious Adverse Event (SAE) is any unfavourable medical occurrence that results in: a) death; b) threat or risk to life; c) hospitalization or extension of a pre-existing hospitalization, excepting elective surgeries and hospitalizations expressed in the protocol; d) persistent or significant disability; e) congenital anomaly or birth defect; f) significant medical occurrence which, based on appropriate medical judgment, may harm the patient and/or require medical or surgical intervention to prevent any other occurrences mentioned above(CONSELHO NACIONAL DE SAÚDE; MINISTÉRIO DA SAÚDE, 2003).

The researchers were not required to notify the Research Ethics Committee (CEP) on any adverse event and, thus, there was no temporary suspension to use the supplement or withdrawal of any research subject from the study.

1.10Statistical analysis plan

Data were analysed by using descriptive and analytical statistics according to relative and absolute frequency. Kolmogorov-Smirnov test and histogram inspection were applied to verify the normality of continuous variables. According to type of variable and normality, statistical tests were used to verify the difference between groups.

Among the nominal qualitative variables, the following stand out: gender, race, and nutritional risk. As quantitative variables, the following are explicit: age, height, weight, BMI, total lymphocyte count, and CRP.

Student's t test was used for normal variables and Mann Whitney U test was applied for non-parametric variables through the mean and standard deviation to verify the difference between the comparison groups. Categorical covariates were evaluated by using Pearson's chi-square test.

To quantify the probability of effectiveness for the proposed treatment (CRP reduction and lymphocyte increase in 30% or more), Relative Risk (RR) and Relative Risk Reduction (RRR) were calculated. All relative risk calculations were made in a 2x2 table. For calculating the RR, the p-value was obtained by using Fisher's exact test. For all analyses, a confidence level of 95.0% ($p<0.05$) was adopted. The study power was 99.64%.

Data were organized and analysed by using *Stata/MP 16.0 for Windows*(StataCorp LLC ®, Texas, USA). The software was licensed to State University of Bahia (UNEB) and linked to the Teaching, Research and Extension Laboratory in Public Health (LEPESC/UNEB).

1.11 Ethical aspects

This research project was registered at Research Ethics Committee (CEP/UNEB) on CAAE no. 31801820.0.0000.0057 and approved by opinion no. 4.031.187. The clinical trial was also registered in the Brazilian Registry of Clinical Trials (REBEC) under UTN No. U1111-1252-3270.

At all research stages, Resolution 466/2012 regarding research involving human beings and the principles of the Declaration of Helsinki were respected. The Informed Consent Form, in two copies, was used immediately before collecting the data to the research.

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