**ANAHUAC UNIVERSITY MEXICO-NORTE**

**FACULTY OF HEALTH SCIENCES**

**RESEARCH COORDINATION**

# EFFECT OF A NUTRITIONAL SUPPORT SYSTEM TO INCREASE SURVIVAL AND REDUCE MORTALITY IN PATIENTS WITH COVID-19 IN STAGE III AND COMORBIDITIES —— A BLINDED RANDOMIZED CONTROLLED CLINICAL TRIAL

## Investigation Protocol

Clinical Trials identifier: NCT04507867.

Protocol identification number: 202036.

CONBIOETICA-15-CEI-004-20160729.

**MEXICO CITY, JULY 2020**

## 1. ABSTRACT

The disease caused by SARS-CoV-2 has become a pandemic in which its evolution and complications depend on the immunological capacity of the host. In Mexico, the global lethality rate of the virus is 11.3% (1,2), with a mortality rate of 0.2795 per 1000 inhabitants (3). It is relevant to study therapies that allow the host to recover and reduce complications; to date, the virus has been characterized by an inflammatory cascade, increased by the overproduction of cytokines, the decrease of metalloenzymes and the rapid dissemination of the virus. There are several lines of treatment, however, nutrition is considered as a caloric contribution and not as part of the treatment. For this reason, this study will evaluate the evolution of patients with COVID-19 assisted by a nutritional support system (NSS) based on supplements such as omega 3, zinc, selenium, vitamin D, glutamine, arginine, magnesium, cobalamin, pyridoxine, thiamine, prebiotics, probiotics, foods such as Spirulina Maxima, brewer's yeast and the effect of this therapy in the reduction of complications and comorbidities. Research question: Will the nutritional support system reduce complications in stage III COVID-19 patients with comorbidities (type 2 DM, HAS, overweight/obesity with BMI <35) with a better benefit than that achieved with conventional nutritional therapy? Hypothesis: The nutritional support system will reduce complications in patients with COVID-19 with stage III comorbidities. General Objective: To determine the effect of the use of a nutritional support system on complications in patients with COVID-19 and stage III comorbidities. Methodology: A controlled, blinded and randomized clinical trial will be conducted in patients with COVID-19, both sexes, older than 30 years old, hospitalized at Centro Médico ISSEMYM Toluca Arturo Montiel Rojas, who meet the inclusion criteria and are classified in stage III. Two groups will be evaluated: 1. Control group (CG) which will receive the normal diet implemented by the hospital and 2. Intervention group (IG) which will receive the normal diet implemented by the hospital together with the NSS. Blood chemistry of 46 elements, coagulation tests, blood biometry, % SpO2, homocysteine, 25-hydroxycholecalciferol, clinical data and anthropometry will be evaluated at baseline and during their hospital stay.

## 2. THEORETICAL FRAMEWORK

### 2.1 Theoretical background

**Coronaviruses are a group of RNA (ribonucleic acid) viruses that cause respiratory and digestive diseases, producing mild to severe symptoms such as acute respiratory distress syndrome and death. (4,5,6) SARS-CoV-2 virus can cause the disease called coronavirus disease 2019 (COVID-19), first manifested in Wuhan, China, in December 2019. (7,8)**

### 2.2 Epidemiology

**Worldwide, 11,874,226 confirmed cases and 545,481 deaths have been reported. In Mexico, 282,283 cases have been confirmed and 33,526 deaths. The global case fatality rate is 4.6%, in Mexico it is 11.3% with a mortality rate of 0.2795 per 1000 population (2,3,7).**

### 2.3 Stratification

**In a retrospective study conducted in January 2020 in Beijing, the classification of COVID-19 was described as: mild, severe, non-pneumonic and asymptomatic, depending on the clinical manifestations and radiological evidence of pneumonic process (7,8). The presence of comorbidities such as obesity, cardiovascular disease, arterial hypertension, type 2 diabetes mellitus and immune deficiency states have been associated with more severe disease (9,10). In Mexico, according to the interim algorithms for the care of COVID-19 are classified according to the time of evolution, signs and symptoms, the application of different clinical scales and laboratory findings in: stage I (early infection) outpatient management, stage II (pulmonary phase) medical review and evaluation for hospitalization; and stage III (hyperinflammatory phase, cytokine storm) the patient requires hospitalization in reconversion areas. (11)**

### 2.2 Transmission and Pathophysiology

**Transmission occurs through the air or through fomites; airborne spread is due to respiratory droplets reaching 1-2 m generated by talking, coughing and sneezing; the virus can remain viable for 24 to 72 hours on surfaces, which allows contagion by contact with these fomites and touching eyes, nose or mouth (9); SARS-CoV-2 enters human cells**

**through the respiratory tract by binding to angiotensin-converting enzyme 2 (ACE2), expressed in alveolar cells, cardiac myocytes, vascular endothelium and other cells (13,14,15). Infection occurs in 3 steps: binding to the receptor, conformational changes in glycoprotein S and proteolysis of cathepsin L within the endosomes. The virus, upon entering the host cell, releases the RNA genome into the cytoplasm, subsequently attaches to ribosomes and gives way to viral genome translation. SARS-CoV-2 can affect different cellular levels (16,17). ACE2 expressed in the epithelial cells of the esophagus and enterocytes in ileum and colon causes gastrointestinal colonization in patients infected by COVID-19. Viral infections are related to dysbiosis due to alterations in the intestinal microbiota, immunological defects or different pathologies that cause damage to the Peyer's plaques, decreasing the production of T17 lymphocytes, generating a state of immunosuppression. These effects are reversible after adequate probiotic colonization (gastrointestinal microbiota), as well as the supply and metabolism of different micronutrients such as vitamins D and A that modulate the function of neutrophils and participate in the differentiation of T lymphocytes to Th1 or Th17. Dysbiosis inhibits the synthesis of short-chain fatty acids, which influence intracellular and extracellular immunoregulatory signaling, altering gene transcription, promoting antibody synthesis, cell differentiation, among others, which has effects on distal organs such as the lungs, comprising the microbiota-intestine-lung axis. (18,19,20)**

### 2.3 Clinical manifestations

The clinical manifestations of COVID-19 are nonspecific, may remain asymptomatic, mild clinical manifestations such as fever, cough, sore throat, fatigue, dyspnea, myalgia, headache, vomiting and diarrhea (14,20). Presentation and severity usually vary by age (> 65 years), comorbidities and compromised immune system. In these cases the patient may be at increased risk of developing organ dysfunction, including shock, acute respiratory distress syndrome (ARDS), acute cardiac injury, and acute kidney injury, resulting in a higher mortality rate (15). Symptoms of COVID-19 infection appear after an incubation period of 5-6 days, the time period between symptom onset and death ranges from 6 to 41 days, with a median of 14 days. The median time from symptom onset to dyspnea is 5 days, hospitalization 7 days and the onset of ARDS is 8 days. The need for admission to the Intensive Care Unit (ICU) in several published series is 25 - 30%. Different complications have been evidenced, which include (21):

**2.3.1 Pulmonary damage:** in severe cases of COVID-19 there is a decreased ratio between arterial oxygen partial pressure and fractional inspired oxygen (PaO2: FiO2 ratio) with concomitant hypoxia and tachypnea. In addition, low CO2 levels have been observed as the mean partial pressure of carbon dioxide (PaCO2) level at 34 mmHg. In summary, hypoxia and hypercapnia are observed in severe cases. (22). IL-6 acts as a potent proinflammatory factor and has been implicated as a strong predictor of respiratory failure (23).

**2.3.2 Immune response:** Damage by immune mechanisms is caused by the storm of cytokines, mainly inflammatory, which activate T lymphocytes, macrophages and endothelial cells, with the consequent vascular permeability, activation of the complement cascade and coagulation (24,25). In patients with severe COVID-19, there is a decrease in the absolute number of circulating CD4+ cells, CD8+ cells, B cells and NK cells; a decrease in monocytes, eosinophils and basophils has also been reported. (26) SARS-Cov-2 requires in a key way the association with S-adenosyl-L-methionine (SAM), with its nsp 14N7-Mtase regions. When SAM is transformed into S-adenosylhomocysteine (SAH) and this is in contact with SAH hydrolase (SAHH), homocysteine is obtained, which is an intermediate that stimulates oxidative stress and is recycled by remethylation and trans-sulfuration in humans. In a study published in the journal Nature Communications, the focus was on understanding the homocysteine-related regulatory mechanisms that stimulate the angiotensin II receptor type. Thus, SARS-CoV-2 utilizes increased activation of ACE2 (27).

**2.3.3 Gastrointestinal symptoms:** Studies have identified SARS-CoV-2 RNA (obtained by PCR) in stool samples from infected patients, and it was found that the ACE2 receptor is expressed in enterocytes, suggesting that it can infect and actively replicate in the gastrointestinal tract (evidenced by electron microscopy), which has implications for the management, transmission (fecal-oral) and control of COVID-19. Some patients reported symptoms such as diarrhea, vomiting, and abdominal pain during the course of the disease. (28)

**2.3.4 Nutritional status:** Lymphopenia, which is a marker of malnutrition, is a poor prognostic factor in patients with COVID-19. Circulating albumin levels should not be considered as a nutritional marker in patients with active inflammatory response, since it was recently reported that low levels of prealbumin predict progression of ARDS (29). The acute phase response, induced by inflammation and infections, alters lipid metabolism, decreasing serum HDL and increasing triglycerides, which initially may help fight infection, but increases the risk of

atherosclerosis. Reducing inflammation has been shown to result in a return of the lipid profile to normal. (30,31) It has been shown that in patients with muscle wasting due to disease, the use of glutamine, arginine and HMB (beta hydroxy beta methylbutyric acid) can decrease muscle loss because they can promote the production and decrease in protein degradation. Since arginine improves cell size and protein synthesis and glutamine activates cell division.

**2.3.5 Thrombotic and thromboembolic complications:** the main hemostatic complications include mild thrombocytopenia (<100. 000/µL) and increased D-dimer levels, which are associated with a high risk of requiring mechanical ventilation, admission to the ICU or death. Prolongation of prothrombin time (PT), thrombin time (TT), tendency of shortening of activated partial thromboplastin time (aPTT), increased INR (international normalized ratio) and high fibrinogen levels have also been associated. This predisposes to thrombotic events; whether these alterations are specific to SARS-CoV-2 or are a consequence of the cytokine storm that precipitates the onset of systemic inflammatory response syndrome (SIRS) as seen in other viral diseases remains unknown. Another consideration that has not been investigated is that hemostatic changes are related to liver dysfunction. (12)

**2.3.6 Kidney manifestations:** The alterations associated to COVID-19 have been hematuria, acute kidney injury (AKI), the most common finding is proteinuria that goes from mild to moderate; it has also been evidenced that podocytes and tubular epithelial cells are infected. Diao et al. found that SARS-CoV-2 antigens agglomerate in renal epithelial tubules, i.e., the human kidney is directly infected causing renal dysfunction and collaborating with viral propagation in the body (32). It has been evidenced that acute renal injury is related to higher mortality. (33,34)

**2.3.7 Metabolic control:** It is estimated that 20-50% of infected patients in the world have diabetes, as a consequence of chronic inflammatory levels such as obesity. Mexico ranks first in childhood obesity and second in adult obesity, which makes its population a population with a high potential for SARS-COVID-2 complications, with obesity being the most important risk factor regardless of age. The comorbidities of obesity are hypertension, diabetes and coronary heart disease, (35,36) conditions that together aggravate the cellular inflammatory state. Obesity, overweight and metabolic syndrome alter the innate immune response altering the first lines of defense, the elevation of IL-6 caused by obesity also participates in the pathogenesis of pulmonary diseases, asthma, respiratory distress and hepatic damage. Glycoproteins such as DPP4 (dipeptidyl peptidase 4) are found in epithelial cells of the

respiratory tract, intestine, capillaries and myocardium, being important for angiogenesis, cardiovascular regulation and inflammation, an increased expression of DPP4 is associated with obesity and metabolic syndrome (37). Chronic inflammatory states develop peripheral leptin resistance causing dysregulation of insulin and glucose sensitivity (38). SARS-CoV-2 is thought to infect islet cells via the ACE2 receptor, impairing islet function and altering the balance of blood glucose metabolism (39). It has been seen that viral diseases can cause dyslipidemia, recent studies refer that the reduction of lipid levels is related to the severity of symptoms, the theories are: 1) SARS-CoV-2 can impair liver function, reducing LDL-c (low density lipoprotein concentration) biosynthesis; 2) acute inflammation alters lipid metabolism, mainly by TNF-a, IL-6 and IL-1b ; 3) lipids are sensitive to degradation by free radicals, which are elevated in host cells with viral infection; 4) altered vascular permeability can cause leakage of cholesterol molecules into tissues (alveolar spaces) and form exudates. (40)

**2.3.8 Hepatic:** Liver injury is more common in severe cases of COVID-19, it may be directly caused by viral infection of liver cells, those patients with liver comorbidities showed abnormal levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as the disease progressed. In addition, it has been proposed that ACE2 receptor expression is increased in cholangiocytes, suggesting that SARS-CoV-2 may cause destabilization of liver function through its direct binding to ACE2-positive cholangiocytes. Another factor that may be contributing to liver injury is cytokine storm and pneumonia-related hypoxia. (41)

### 2.4 General treatment

Treatment is supportive and symptomatic, depending on the severity of symptoms, although there is no specific treatment so far, most patients will require supplemental oxygen. Pharmacological treatment varies depending on the complications and the patient's clinical condition, including medications such as Ceftriaxone, Azithromycin, Oseltamivir and Ivermectin; together with the nutritional therapy recommended by the WHO, calculating the energy requirement per weight of 27 - 30 kcal/kg/day; protein requirement up to 1.5 g/kg/day in case of not having chronic renal failure; carbohydrate and lipid requirement with a range of 30:70 in patients without respiratory failure and 50:50 in patients with respiratory failure. (42, 43)

### 2.5 Nutritional Support System (NSS)

Nutritional status influences the outcome of patients with COVID-19, but so far there is no information on the impact of early nutritional support in patients with COVID-19 before ICU (28). Zhang and Liu recently developed a list of nutrients with potential anticoronavirus effects based on in vitro and clinical studies. However, none of the literature available so far has discussed how to adapt currently available guidelines for nutritional therapy during illness to the specific clinical scenario of COVID-19 (13).

**2.6 Evidence for specific nutrients**

Probiotics are beneficial in the prevention of acute upper respiratory tract infection, helping to counteract dysbiosis in patients with COVID-19. Specifically, Saccharomyces Boulardii, also increases IgA levels in the intestine reducing the proinflammatory response, (44). On the other hand, beta-glucans from the cell wall of brewer's yeast (Saccharomyces Cerevisiae) stimulate the immune system, especially those related to inflammatory responses (leukocytes and epithelial cells) and the reticuloendothelial system (45). Nutrients may favor the immune response with respect to the common characteristics currently presented in patients with COVID-19 (16). The SARS-CoV-2 viral genome encodes for the nsp12 protein, which harbors RNA-dependent RNA polymerase (RdRP) activity. It has been shown that vitamin B12 (methylcobalamin) can bind to the active site of the nsp12 protein, preventing association with RNA and thereby inhibiting the RdRP activity of nsp12 (46). On the other hand, administration 2-500 μM of Zinc significantly inhibits influenza replication in vitro, through inhibition of RNA-dependent RNA polymerase (RdRp), with a similar effect occurring with SARS-CoV, where binding and elongation of the RdRp template is inhibited (47). In addition, C-phycocyanin, an extract found in Spirulina maxima, acts by blocking hemagglutination of virus particles to inhibit influenza virus strains and may help prevent morbidity in airway diseases (48). Protein D1, derived from Omega 3, reduces virus replication of some strains of influenza viruses, so omega 3 is also considered an important nutrient when considering a feeding scheme with antiviral action (49). Arginine and resveratrol are able to decrease apoptosis induced by MERS-CoV and inhibit infection caused by it, prolonging cell survival after infection (50). These antiviral mechanisms are due to the fact that resveratrol activates the ERK 1/2 signaling pathway and promotes cell proliferation by enhancing SIR1 signaling, both of which are related to cell survival and DNA repair in response to DNA damage (51). The release of proinflammatory cytokines such as TNFα, IL-6, IL-8 and IFN (interferon Alpha, Beta and Gamma) correlates with disease severity. Administration of vitamin D especially in its active form 1, 25, dihydroxycholecalciferol [1, 25 (OH) 2D3], causes modulation in the immune

system and antiviral activity by reducing the expression of proinflammatory cytokines (TNFα, IL-6, IL-8) and increasing the expression of anti-inflammatory cytokines (IL 4, IL 10) by macrophages through regulation by vitamin C (52). Khare et al (53) observed that treatment of human lung epithelial cells with 100 or 30 nM of 1α, 25 (OH) cholecalciferol before or after H1N1 exposure significantly decreased TNFα, IFNβ and IFNγ levels and IL-8 and IL-6 levels. Vitamin D deficiency (plasma 25 (OH) D levels <50 nmol / L) has been identified in patients with acute respiratory distress syndrome (ARDS) (54).

25-hydroxycholecalciferol is a stable marker of vitamin D status that can be measured in blood. This vitamin immunomodulates the innate and adaptive immune system, which is useful against the cytokine storm caused by SARS-Cov-2, since its active metabolites modify NF-κB signaling in macrophages, reduces the synthesis of proinflammatory mediators (TNFα, IL -6 and MCP-1) and decreases the recruitment of monocytes and macrophages, lowering overall tissue inflammation, in addition, it has been documented to have an antifibrotic effect in lung tissue. (55,56, annex 4). Omega 3, derived in the formation of docosahexaenoic acid (DHA), reduces extremely high inflammatory parameters, since it regulates lymphocyte proliferation, cytokine production and neutrophil chemotaxis (57). Folates mediate the production of proinflammatory cytokines and the immune response mediated by Th-1 cytokines. Therefore, their deficiency decreases the antibody response to various antigens, including the blastogenic response of T lymphocytes to certain mitogens, and the capacity of CD8 T lymphocyte cells (58). There is evidence that magnesium plays a key role in the immune response as a cofactor for immunoglobulin synthesis, (48) as well as a relationship between magnesium deficiency and increases in proinflammatory cytokines (59). It is known that intracellular free glutamine in skeletal muscle is markedly reduced in the stress response to surgery, trauma and inflammatory states; thus, reduced plasma glutamine values have been detected in hospitalized patients with emphysema, COPD and evidence of muscle wasting (60). SARS-CoV-2 enters the cell through its surface proteins; however, this protein must be divided into 2 domains, where furin intervenes. It has been shown that folic acid inhibits furin activity, preventing the virus from entering the cell. (61)

## 3. JUSTIFICATION

COVID-19 is a disease that spreads rapidly and has a high mortality rate, which has caused the collapse of health systems; therefore, it is important to implement a comprehensive treatment to avoid complications of the disease, reduce the length of hospital stay and provide a rapid recovery. Treatment costs can vary from $500,000 Mexican pesos per admission to $1,512,000

pesos, considering an average hospital stay of 12 days (5). It has been seen that 26.4% of hospitalized patients recovered and were discharged from the hospital, and 46.4% improved and were reclassified. 13.4% have died (6). There are several lines of treatment, but nutrition is only considered within the caloric intake. In this study a nutritional therapy called NSS will be evaluated, trying to reduce complications in patients with COVID-19, as well as reduction of hospital stay days, disease progression, percentage of progression to mechanical ventilation and mortality. This study will provide scientific evidence to establish new strategies to improve the clinical evolution of patients with COVID-19 worldwide. This study is feasible given that it has the material and human resources to be carried out and is pertinent given that it seeks to contribute to the study of new nutritional therapies to reduce complications and mortality in patients with COVID-19.

## 4. STATEMENT OF THE PROBLEM

To date, cases of COVID-19 have been reported in 212 countries. It is currently known that COVID-19 mainly affects the respiratory system, although it is not the only one; it can cause mild to severe symptoms such as pneumonia, multiple organ failure or even death. It has a case fatality rate of 11.3% in Mexico. No pharmacological treatment has proven to be totally effective, therefore, the main treatment is symptomatic and supportive (62). Conditions such as age (>65 years), presence of comorbidities such as overweight or obesity, chronic pulmonary diseases (asthma, COPD), cardiovascular diseases, systemic arterial hypertension (SAH), diabetes (DM type 2) and immune deficiency states have been associated with more severe disease progression. New evidence suggests a possible regulatory role of specific nutrients in the pathogenesis and development of COVID-19 complications, but so far no nutritional support system has been integrated as part of the therapeutics. This proposal seeks to help patients reduce the appearance of complications and avoid their admission to the ICU. Recent research supports that certain nutrients favor the immune response with respect to the common characteristics that are currently being presented in patients with COVID-19. The present study proposes an evidence-based nutritional support scheme for the comprehensive management of patients with COVID-19. It is expected that this system will act synergistically with the current treatment and improve the evolution of the patients.

## 5. RESEARCH QUESTION

Will the nutritional support system reduce complications in patients with COVID-19 and comorbidities (type 2 DM, HAS, overweight/obesity with BMI <35) in stage III, with a better benefit than that achieved with conventional nutritional treatment?

## 6. HYPOTHESIS

The nutritional support system will reduce complications in patients with COVID-19 and comorbidities (type 2 DM, HAS, overweight/obese with BMI <35) in stage III, with a better benefit than that achieved with conventional nutritional treatment.

## 7. GENERAL OBJECTIVE

To determine the effect of the use of a nutritional support system on complications in patients with COVID-19 and comorbidities (type 2 DM, HAS, overweight/obesity with BMI <35) in stage III, compared with conventional nutritional treatment.

### 7.1. SPECIFIC OBJECTIVES

1. To correlate biochemical and inflammatory factors with the evolution of the study groups.

2. To correlate body composition with the clinical evolution of the patients.

3. To evaluate the days of hospital stay, disease progression, percentage of progression to ventilator and mortality as predictive indexes of patient improvement.

4. To stratify in groups by age ranges (30-39, 40-49, 50-59 and >60 years).

## 8. METHODOLOGY

**8.1** Type of study and overall design: Blinded, randomized controlled clinical trial.

8.2 Study Universe.

Patients diagnosed with COVID-19 by PCR test, who are attended at Centro Medico ISSEMYM Toluca Arturo Montiel Rojas, located at Paseo Tollocan, Av. Baja Velocidad km 575, Barrio de Sta Clara, Toluca de Lerdo, State of Mexico; both sexes, with presence of comorbidities and in stage III.

8.3 Selection and sample size: the sample size will be calculated using the formula: $n = \frac{z^2pq}{e^2}$, where: 95% confidence level (z = 1.96), p = 0.975, q = 0.025, with a margin of error of 5% (e = 0.05), obtaining 38 patients per group for the sample.

8.4 Inclusion and exclusion criteria

Inclusion criteria: 1. Patients admitted to the Centro Medico ISSEMYM Toluca Arturo Montiel Rojas, diagnosed with COVID-19. 2. Patients in need of supplemental O2 with nasal prongs or mask-reservoir due to satO2 <90% and respiratory distress. 3. With concomitant diseases such as cardiovascular disease, diabetes mellitus 2, hypertension, overweight or obesity BMI <35. 4. 5. Over 30 years of age. 6. Tolerant of oral feeding. 7. Signed informed consent letter.

Non-inclusion criteria: 1. Who requires mechanical ventilatory support. 2. 2. Present neurological or neurodevelopmental disorders. Severe gastroesophageal reflux and/or dysphagia. 4. 4. Patients with any type of surgery performed in a period of less than 3 months. 5. Allergies to any ingredient of the study treatment. 6. Who present acute malnutrition. 7. Patients who require admission to the Intensive Care Unit. 8. Who has criteria of sepsis or septic shock. 9. Glomerular filtration rate less than 60ml/min/1.73m$^2$.

Criteria for elimination: 1. Treatment failure. 2. Admission to ICU for any reason. 3. Patients who do not tolerate the oral route. 4. Reactions to treatment that compromise the health of the patients.

Sampling and allocation: Consecutive cases. Systematic randomized allocation by means of a sequence of random numbers constructed with the Excel program divided into two groups.

8.4 Procedure

Once the participants have been selected, the patients and/or family members will be interviewed to explain the protocol and to obtain the signatures of the informed consent letters (Annex 1). Confirmatory test for COVID-19 by PCR, which should confirm the diagnosis in order for the study to continue. Complete clinical and dietary history. Complete blood count: leukocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, erythrocytes, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean hemoglobin concentration (MHC), mean corpuscular hemoglobin concentration (MCHC), platelets and mean platelet volume (MPV). 4. Blood chemistry (glucose, urea, urea nitrogen (BUN), creatinine, BUN/creatinine ratio, uric acid, calcium, phosphorus, sodium, potassium, chloride, magnesium, glomerular filtration rate (GFR), triglycerides, total cholesterol, low density lipoproteins (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), total lipids, total, direct and indirect bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), total proteins and albumin. 5. D-dimer (see section 2.3.6). 6.

Coagulation times, bleeding time and prothrombin time. (See section 2.3.6). 7. Fibrinogen. (Serum electrolytes: sodium, potassium, magnesium, and chlorine. (See section 2.3.7 and 2.3.8). 9. Serum homocysteine levels. (See section 2.3.3). Serum levels of 25 hydroxycholecalciferol. (See section 2.6). 11. Taking of tricipital fold and arm circumference, % muscle mass, % fat, BMI, weight, height. 12. Assignment to the research group in a random way, which will have the following characteristics: A) QA: calculate the Basal Energy Expenditure (BEE) per 1.5 of stress factor by means of the Harris Benedict equation. The distribution of macronutrients will be 50% for carbohydrates, 30% for lipids and 20% for proteins. The food will be established according to what is established in the nutrition department of the Centro Médico ISSEMYM Toluca Arturo Montiel Rojas. B) GI: calculate the Basal Energy Expenditure (BEE) plus the stress factor using the Harris Benedict equation. The distribution of macronutrients will be 50% for carbohydrates, 30% for lipids and 20% for proteins. The food will be established according to what is established in the nutrition department of the Centro Médico ISSEMYM Toluca Arturo Montiel Rojas, together with the following supplementation: 1. Neurobion injectable solution of 10 mg, 1 every 24 hrs for 5 days IM. 2. One sachet of NSS-1 in the morning and one sachet in the afternoon mixed with 400 ml of water each, containing: Spirulina Maxima 2. 5 g, folic acid 5 mg, Glutamine 5g, Cyanomax Ultra (10 grams of powder), ascorbic acid 1 g, zinc 20 mg, selenium 100 mcg, cholecalciferol 2000 IU, resveratrol 200 mg, concentrated omega 3 fatty acids (10 grams of powder), L-Arginine 1.5 g, and magnesium 400 mg. During the entire procedure. 3. 500 mg of *Saccharomyces Bourllardii*, administered with 1 capsule of 250 mg during breakfast and lunch, for the first 6 days.

The energy balance of the supplements will be considered within the designated caloric balance for this group. The follow-up will be performed daily for 21 days or earlier, if they are discharged from hospital due to improvement in evolution or require access to the ICU, at which time the patient concludes the study. The following points will be supervised: 1. Application and consumption of the NSS supplementation as appropriate. 2. Recording of all the information in the records. 3. Food diaries of each patient. 4. Laboratory studies: QS every other day, BH every 3rd day, coagulation times every 3rd day, D-dimer and fibrinogen every 3rd day, serum electrolytes every other day, serum homocysteine every other day and serum 25 hydroxycholecalciferol levels every other day. 5. SpO2 %, heart rate, respiratory rate. 6. Clinical evaluation and record of bowel movements. 7. Taking of tricipital fold and arm

circumference, % muscle mass, % fat, BMI, weight, height every two days. 8. Days of hospital stay.

## 9. ETHICAL CONSIDERATIONS

The present clinical trial complies with the guidelines stipulated in the regulations of the General Health Law on Research, article 17 of which considers it a minimum risk research. According to what is established in articles 36 and 37 of this Law, the consent must be obligatorily in writing by means of a letter of informed consent and a letter of agreement (annex 1). This letter will be signed voluntarily by the patient. The study does not affect the living, moral or physical conditions of the study subjects. It does not violate the basic principles of the Declaration of Helsinki, the Nuremberg Code and the NOM-012-SSA3-2012 about scientific research in humans, it also adheres to the international Ethical Guidelines for biomedical research involving human subjects.

## 10. FUNDING

In order to carry out this research it is necessary to have working and protection equipment, which has an estimated cost of $348,082.50 pesos MN. The supplementation of the NSS has an approximate total cost of $976,320.00 pesos MN. The evaluation and diagnostic methods will be the same as those routinely performed at the hospital. This project, once authorized, will be submitted to the Universidad Anahuac Mexico Norte for institutional budgeting and will receive support from the private sector.

## 11. BIBLIOGRAPHIC REFERENCES

1. OMS. Situation Report-113 al 12 de mayo de 2020. Disponible en: https ://www.who.int/docs/default-source/coronaviruse/situation-reports/20200512-covid-19-sitrep113.pdf?sfvrsn=feac3b6d_2
2. Gobierno de México. Comunicado Técnico Diario COVID-19 MÉXICO 11 de mayo 2020. Disponible en: https://www.gob.mx/salud/documentos/coronavirus-covid-19-comunicado-tecnico-diario-238449
3. Gobierno de México. Comunicado Técnico Diario COVID-19 MÉXICO 09 de julio 2020. Disponible en: https://www.gob.mx/coronavirus.gob.mx/2020/07/09conferencia-9dejulio/
4. 1 Valentín, E. L., Montero, J. S. N., & Florentini, M. G. Q. (2020). Coronavirus causante del síndrome respiratorio de Oriente Medio (MERS-CoV). Revista Médica Carriónica, 1(1).
5. Aragón-Nogales, R., Vargas-Almanza, I., & Miranda-Novales, M. G. (2019). COVID-19 por SARS-CoV-2: la nueva emergencia de salud. Rev Mex Pediatr, 86(6), 213-218.
6. Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S. C., & Di Napoli, R. (2020). Features, evaluation and treatment coronavirus (COVID-19). In Statpearls [internet]. StatPearls Publishing.
7. Tian S, Hu N, Lou J, Chen K, Kang X, Xiang Z, et al. Characteristics of COVID-19 infection in Beijing. J Infect. 2020;80(4):401-6.
8. OMS. Situation Report-111 al 11 de mayo de 2020. Disponible en: https://www.who.int/docs/defaultsource/coronaviruse/situation-reports/20200511- covid-19-sitrep-112.pdf?sfvrsn=813f2669_2
9. Wang Y, Liu, Liu, Wang X, Luo N, Li L. Clinical Outcomes in 55 patients With Severe Acute Respiratory Syndrome Coronavirus 2 Who Were Asyntomatic at Hospital Admission in Shenzhen, China. J Infect Dis. 2020;221(11):1770-4.

10. Bonanad, C., García-Blas, S., Tarazona-Santabalbina, F. J., Díez-Villanueva, P., Ayesta, A., Forés, J. S., ... & Martínez-Sellés, M. (2020). Coronavirus: la emergencia geriátrica de 2020. Documento conjunto de la Sección de Cardiología Geriátrica de la Sociedad Española de Cardiología y la Sociedad Española de Geriatría y Gerontología. Revista Española de Cardiología.
11. Algoritmos interinos para la atención del COVID-19 [internet]. Ciudad de México: IMSS; 2020 [citado 14 Julio 2020]. Disponible en: https://saluddigital.com/wp-content/uploads/2020/05Algoritmos-IMSS.pdf
12. Bikdeli B, Madhavan MV, Jimenez D, Chuich T, Dreyfus I, Driggin E, et al. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(23):2950-73.
13. Liu Y, Zhang L. Potential interventions for novel coronavirus in China: A systematic review. Journal of Medical Virology. 2020; 92(5):479-490
14. OMS. WHO characterizes COVID-19 as a pandemic. Disponible en: https://www.paho.org/hq/index.php?option=com_content&view=article&id=15756:who-characterizes-covid-19-as-a-pandemic&Itemid=1926&lang=es
15. Chen N, Zhou M, Dong X, Qu j, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10222):391-93
16. Zhang, L., & Liu, Y. (2020). Potential interventions for novel coronavirus in China: a systemic review. Journal of medical virology.
17. Anand S, Mande SS. Diet, Microbiota and Gut-Lung Connection. Frontiers in Microbiology. 2018;9(2147).
18. Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J., Pfeffer, M. A., & Solomon, S. D. (2020). Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. New England Journal of Medicine, 382(17), 1653-1659.
19. Morais AHA, Passos TS, Maciel BLL, da Silva-Maia JK. Can Probiotics and Diet Promote Beneficial Immune Modulation and Purine Control in Coronavirus Infection? Nutrients. 2020;12(6).
20. Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr. 2020;87(4):281-6.
21. VALENCIANA, C. PROCEDIMIENTO DE ACTUACIÓN FRENTE A CASOS DE INFECCIÓN POR EL NUEVO CORONAVIRUS (SARS-CoV-2). Disponible en: https://www.redaccionmedica.com/contenido/images/Procedimiento_COVID_19%281%29.pdf
22. Geier MR, Geier DA. Respiratory conditions in coronavirus disease 2019 (COVID-19): Important considerations regarding novel treatment strategies to reduce mortality. Med Hypotheses. 2020;140:109760.
23. Li J, Wang X, Chen J, Zuo X, Zhang H, Deng A. COVID-19 infection may cause ketosis and ketoacidosis. Diabetes Obes Metab. 2020.
24. Chhetri S, Khamis F, Pandak N, Al Khalili H, Said E, Petersen E. A fatal case of COVID-19 due to metabolic acidosis following dysregulate inflammatory response (cytokine storm). IDCases. 21: © 2020 The Authors.; 2020. p. e00829.
25. Infusino F, Marazzato M, Mancone M, Fedele F, Mastroianni CM, Severino P, et al. Diet Supplementation, Probiotics, and Nutraceuticals in SARS-CoV-2 Infection: A Scoping Review. Nutrients. 2020;12(6).
26. Ahmad I, Rathore FA. Neurological manifestations and complications of COVID-19: A literature review. J Clin Neurosci. 2020;77:8-12.
27. Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduction and Targeted Therapy. 2020;5(1):84.
28. Singh Y, Gupta G, Kazmi I, Al-Abbasi FA, Negi P, Chellappan DK, et al. SARS CoV-2 aggravates cellular metabolism mediated complications in COVID-19 infection. Dermatol Ther. 2020:e13871.
29. Wong SH, Lui RN, Sung JJ. Covid-19 and the digestive system. J Gastroenterol Hepatol. 2020;35(5):744-8.
30. Feingold KR, Grunfeld C. The Effect of Inflammation and Infection on Lipids and Lipoproteins. [Updated 2019 Jan 8]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326741/
31. Feingold KR, Grunfeld C. The acute phase response inhibits reverse cholesterol transport. J Lipid Res. 2010;51(4):682-4.
32. Laviano A, Koverech A, Zanetti M. Nutrition support in the time of SARS-CoV-2 (COVID-19). Nutrition. 2020;74:110834.
33. Martinez-Rojas MA, Vega-Vega O, Bobadilla NA. Is the kidney a target of SARS-CoV-2? Am J Physiol Renal Physiol. 2020;318(6):F1454-f62.
34. Lim MA, Pranata R, Huang I, Yonas E, Soeroto AY, Supriyadi R. Multiorgan Failure With Emphasis on Acute Kidney Injury and Severity of COVID-19: Systematic Review and Meta-Analysis. Can J Kidney Health Dis. 2020;7:2054358120938573.
35. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. Int J Obes (Lond). 2020:1-3.

36. Sharma SK, Ghimire A, Radhakrishnan J, Thapa L, Shrestha NR, Paudel N, et al. Prevalence of Hypertension, Obesity, Diabetes, and Metabolic Syndrome in Nepal. International Journal of Hypertension. 2011;2011:821971.
37. Bassendine MF, Bridge SH, McCaughan GW, Gorrell MD. COVID-19 and comorbidities: A role for dipeptidyl peptidase 4 (DPP4) in disease severity? J Diabetes. 2020.
38. Sáinz N, Barrenetxe J, Moreno-Aliaga MJ, Martínez JA. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism. 2015;64(1):35-46.
39. Deng M, Jiang L, Ren Y, Liao J. Can We Reduce Mortality of COVID-19 if We do Better in Glucose Control? Med Drug Discov. 7: © 2020 Published by Elsevier B.V.; 2020. p. 100048.
40. Wei X, Zeng W, Su J, Wan H, Yu X, Cao X, et al. Hypolipidemia is associated with the severity of COVID-19. J Clin Lipidol. 2020;14(3):297-304.
41. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5(5):428-30.
42. Brugliera L, Spina A, Castellazzi P, Cimino P, Arcuri P, Negro A, et al. Nutritional management of COVID-19 patients in a rehabilitation unit. Eur J Clin Nutr. 2020;74(6):860-3.
43. Caccialanza R, Laviano A, Lobascio F, Montagna E, Bruno R, Ludovisi S, et al. Early nutritional supplementation in non-critically ill patients hospitalized for the 2019 novel coronavirus disease (COVID-19): Rationale and feasibility of a shared pragmatic protocol. Nutrition. 2020;74:110835.
44. Thomas S, Przesding I, Metzke D, et al. Saccharomyces boulardii inhibits lipopolysaccharide-induced activation of human dentritic cells and T cell proliferation. Clin Exp Immunol. 2009;156:78-87
45. Castro, M., & de Souza Rodriguez, F. Levaduras: probióticos y prebióticos que mejoran la producción animal. Ciencia y Tecnología Agropecuaria. 2005. 6(1): 26-38.
46. Narayanan N, Nair DT. Vitamin B12 May Inhibit RNA-Dependent-RNA Polymerase Activity of nsp12 from the SARS-CoV-2 Virus. Preprints 2020, 2020030347 (doi: 10.20944/preprints202003.0347.v1).
47. te Velthuis AJW, van den Worm SHE, Sims AC, Baric RS, Snijder EJ, et al. (2010) Zn2+ Inhibits Coronavirus and Arterivirus RNA Polymerase Activity In Vitro and Zinc Ionophores Block the Replication of These Viruses in Cell Culture. PLoS Pathog 6(11): e1001176. Disponible en: https://journals.plos.org/plospathogens/article/file?id=10.1371/journal.ppat.1001176&type=printable
48. Masuda K, Chitundu M (2019) Multiple micronutrient supplementation using spirulina platensis and infant growth, morbidity, and motor development: Evidence from a randomized trial in Zambia. PLoS ONE 14(2): e0211693.
49. Morita, M., Kuba, K., Ichikawa, A., Nakayama, M., Katahira, J., Iwamoto, R., & Kadowaki, A. (2013). The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell, 153(1), 112-125.
50. Chafekar, A., & Fielding, B. C. MERS-CoV: understanding the latest human coronavirus threat. 2018; Viruses, 10(2), 93.
51. Anderson R. The immunostimulatory, antiinflammatory and anti-allergic properties of ascorbate. Adv Nutr Res. 1984;6:19-45.
52. Gruber-Bzura, B. M. (2018). Vitamin D and Influenza—Prevention or Therapy?. International journal of molecular sciences, 19(8), 2419.
53. Dancer, R.C.; Parekh, D.; Lax, S.; D'Souza, V.; Zheng, S.; Bassford, C.R.; Park, D.; Bartis, D.G.; Mahida, R.; Turner, A.M.; et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax 2015, 70, 617–624.
54. Panfili FM, Roversi M, D'Argenio P, Rossi P, Cappa M, Fintini D. Possible role of vitamin D in Covid-19 infection in pediatric population. J Endocrinol Invest. 2020:1-9.
55. Yin K, Agrawal DK. Vitamin D and inflammatory diseases. J Inflamm Res. 2014;7:69-87.
56. Hao Q, Lu Z, Dong BR, Huang CQ, Wu T. 2011. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev 9:CD006895
57. Drake VJ. Immunity In Depth. Linus Pauling Institute. 2016. (Online). Disponible en: https://lpi.oregonstate.edu/mic/health-disease/immunity
58. Tam, M., Gomez, S., Gonzalez-Gross, M., & Marcos, A. (2003). Possible roles of magnesium on the immune system. European journal of clinical nutrition, 57(10), 1193-1197.
59. Engelen M.P., Schols A.M., Does J.D., Deutz N.E., Wouters E.F. Altered glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. Am. J. Respir. Crit. Care Med. 2000;161:98–103. doi: 10.1164/ajrccm.161.1.9901031.m on the immune system. European journal of clinical nutrition, 57(10), 1193-1197.)
60. Zahra S, Maryam Heydari D, Manica N, Mehdi D, Hassan Z, Mohsen M, et al. The Role of Folic Acid in the Management of Respiratory Disease Caused by COVID-192020.
61. Salas-Asencios R, Iannacone-Oliver J, Guillén-Oneeglio, A Tantaléan-Da J, Alvariño-Flores L, Castañeda-Pérez L, Cuellar-Ponce de León L. CORONAVIRUS COVID-19: KNOWING THE CAUSE OF THE PANDEMIC. Biol. 2020;18(1):9–27.

62. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020;382(19):1787-99.
63. Acad. Dr. Raúl Carrillo Esper DPVP. Equilibrio ácido base. Conceptos actuales. Diciembre, 2006 julio, 2020 15 julio, 2020]; XX, Núm 4:[pp 184-92 pp.]. Available from: http://medicasurlomas.com.mx/userfiles/pdf/carrillo/ti064e.pdf.
64. Bustos JM. HIPOXIA Y CIANOSIS (HYPOXIA AND CYANOSIS)Septiembre 2016; 1, núm 9:[pp 9-12 pp.]. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=7070357.
65. Paola TP. Visión panorámica del sistema inmune. Revista Médica Clínica Las Condes. 2012;23(4):446-57.
66. Viada Pupo E, Gómez Robles L, Campaña Marrero IR. Estrés oxidativo. Correo Científico Médico. 2017;21:171-86.
67. Lebish IJ, Moraski RM. Mechanisms of immunomodulation by drugs. Toxicol Pathol. 1987;15(3):338-45.
68. Moctezuma-Velázquez C, Aguirre-Valadez J. [Gastrointestinal and hepatic diseases]. Gac Med Mex. 2016;152 Suppl 1:74-83.
69. Gimeno E. Medidas empleadas para evaluar el estado nutricional. Offarm. 2003;22(3):96-100.
70. M M. Blood Coagulation System Physiology. Hematologia, Fisiología de la hemostasia normal: Agosto, 2017;21, no. 31-42.
71. http://biologia.utalca.cl/wp-content/uploads/2018/01/Agua-y-equilibrio-acido-base.pdf
72. Jasso-Huamán LE, Villena-Pacheco A, Guevara-Linares X. Control metabólico en pacientes diabéticos ambulatorios de un hospital general. Revista Medica Herediana. 2015;26:167-72.
73. Bendezú García RÁ, Casado Martín M, Lázaro Sáez M, Patrón Román GÓ, Gálvez Miras A, Rodríguez Laiz GP, et al. Elevación de las enzimas de función hepática en nuestro medio: estudio etiológico y de la eficacia de una consulta de acto único. Gastroenterología y Hepatología. 2013;36(7):450-6.
74. Castaño Bilbao I, Slon Roblero MF, García-Fernández N. Estudios de función renal: función glomerular y tubular. Análisis de la orina. Nefroplus. 2009;2(1):17-30.
75. López-Romero LA, Romero-Guevara SL, Parra DI, Rojas-Sánchez LZ. ADHERENCIA AL TRATAMIENTO: CONCEPTO Y MEDICIÓN. Hacia la Promoción de la Salud. 2016;21:117-37.
76. REAL ACADEMIA ESPAÑOLA: Diccionario de la lengua española, 23.ª ed., [versión 23.3 en línea]. <https://dle.rae.es> [15 julio, 2020].
77. REAL ACADEMIA ESPAÑOLA: Diccionario de la lengua española, 23.ª ed., [versión 23.3 en línea]. <https://dle.rae.es> [15 julio, 2020].
78. https://www.cancer.gov/espanol/publicaciones/diccionario/def/interaccion-de-medicamentos