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"Nuestra Señora Reina De La Paz"

Campus San Pedro y San Pablo

Protocol:
Safety and Efficacy of Thymic Peptides in the Treatment of Hospitalized COVID-19 Patients in Honduras

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Table of Contents

PROTOCOL

VERSION A

SECTION 1 – TITLE, PURPOSE AND JUSTIFICATION

1.1 Title. Safety and Efficacy of Thymic Peptides in the Treatment of Hospitalized COVID-19 Patients in Honduras.

1.2 Study Purpose. To determine if thymic peptides (Unicahsina) are a new therapeutic option for the treatment of patients infected by COVID-19 in order to improve their clinical prognosis.

1.3 Justification. Coronaviruses are important pathogens in humans and animals, causing respiratory tract and lung parenchymal infections. In late 2019, a novel coronavirus was identified as the cause of a series of pneumonia cases in Wuhan, a city in Hubei province in China (1). The infection spread rapidly, leading to an epidemic throughout China, followed by an increasing number of cases in other countries, affecting all continents except Antarctica (1). WHO declared COVID-19 a pandemic on March 11, 2020 (2). The virus is currently referred to as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the disease caused by the virus is known as COVID-19.

At the time of writing this protocol, more than 5,584,091 cases of COVID-19 have been reported worldwide (3). This is largely due to the ease with which this virus is transmitted. The primary mechanism of spread is person-to-person transmission, which occurs primarily by respiratory droplets (1). Transmission of SARS-CoV-2 via fomites is plausible as it has been shown to remain viable on surfaces for hours and even several days (4).

The coronavirus that causes COVID-19 is a betacoronavirus of the same subgenus as SARS-CoV (5). The incubation period is estimated to be 14 days after exposure, with most cases occurring between 4 and 5 days (6-8). The disease is mild in 81% of cases, severe in 14% and critical in 5% (9). The overall mortality rate is 2.3% (9). The clinical course is characterized by fever (99%), fatigue (70%), dry cough (59%), anorexia (40%), myalgia (35%), dyspnea (31%) and sputum (27%) (10). Pneumonia appears to be the most serious manifestation of infection, characterized mainly by fever, cough, dyspnea and a bilateral pulmonary infiltrate (visible on imaging) (7, 10-12).

Global statistics show that mortality due to COVID-19 increases significantly with age. Pediatric patients under 9 years of age present mild cases with practically zero mortality (13). Between 9 and 39 years of age, statistics indicate a mortality of approximately 0.2% (13). In the age range between 40 and 69 years, the rate oscillates between 1.3 and 3.6% (13). Above 70 years of age, deaths due to COVID-19 increase exponentially, reaching 8% of cases (13). Patients over 80 years of age are the highest risk group with a mortality rate of 14.8% (13).

This remarkable statistical difference in morbidity and mortality is consistent with a biological process that has so far been scarcely addressed: involution of the thymus.

This retrosternal gland is part of the primary lymphoid organs, playing an important role in the maturation and differentiation of T cells to CD4+ and CD8+ subtypes, in addition to secretion of cytokines that mediate the immune response (14). Involution follows a characteristic pattern in which there is a progressive loss of function from the first year of life (15). Between 35 and 45 years of age this process accelerates, losing up to 30% of its function in a decade (15). Finally, after the age of 65 it regresses and is replaced in its totality by adipose tissue (15).

Ferman-Cano et al. suggest that the relative protection observed in the infant population with COVID-19 could be explained by the influence of the thymus. Based on this background and the evident relationship between the age ranges in which thymus involution occurs and the exponential increase in mortality, we propose that at older ages there is a greater deficiency of thymic factors, whose replacement represents a therapeutic mechanism to be explored. In this context, thymic peptides stand out as potent candidates given their immunogenic properties and proven safety.

Thymomodulin is a set of low molecular weight peptides of less than 10,000 Daltons, derived from the acid lysis of calf thymus (16). It has been shown that modulates the maturation and function of T lymphocytes and favors the expression of B cell surface markers (16,17). In vitro, thymomodulin has been shown to possess a myelopoiesis-inducing effect in the bone marrow, stimulating granulocyte and macrophage colonies (18). In addition, in murine studies it has been shown to produce an increase in serum thymic hormone (19).

In clinical studies, thymomodulin has proved to reduce the number of recurrent respiratory infections in children (20). This improvement is associated with a higher CD3 + and CD4 + cell count, increased neutrophilic function and salivary IgA levels (21, 22). It also has multiple reports on its safety and efficacy in patients with immune imbalance, in pathologies such as chronic bronchitis (23-25), bronchial hyperresponsiveness (26), asthma (27-29), food allergy (30, 31), atopic dermatitis, immunosuppression by chemo or radiotherapy for neoplasms (32, 33), initial phases of AIDS (34-36) and senectitude thymic involution (19,37,38).

In geriatric patients, studies prove safety at high doses (19), an increase in the number of lymphocytes, monocytes, IgA, IgG and IgM levels (37-40). Likewise, thymomodulin increases the production of free radicals due to alveolar macrophages (23). It is noteworthy to mention that it can even prevent and emend leukopenia in patients undergoing chemotherapy and radiotherapy (41), which has been proven in patients with breast and genitourinary tract cancer (42).

In studies of acute and chronic toxicity, it has been shown that thymomodulin does not possess teratogenic or embryotoxic effects (43). For this reason, it has a high potential for use during pregnancy.

Given the fact that the population most at risk of presenting severe disease and a higher mortality rate due to COVID-19 are immunosuppressed patients, older adults and/or those with comorbidities, effective therapeutic options with high safety profiles are needed. Currently there are no treatments available that meet these requirements for disease management. Treatment options include protocols using chloroquine or hydroxychloroquine. The evidence in favor of its use remains

controversial, since a number of adverse effects have been documented that could affect the most vulnerable population. They are contraindicated in patients with retinopathy (44), neuromyopathy and heart disease due to the possible increase in the QT interval (45).

Scientific evidence proves the absence of short or long-term adverse effects of thymomodulin, obtaining significant results in the treatment of patients with immunological vulnerability in various respiratory pathologies (23). Taking this into account, it can be considered a promising alternative to combat the COVID-19 pandemic. The following sections will discuss the methodology of the clinical trial that will be used to evaluate its efficacy as a therapeutic option.

Hypothesis

Oral administration of thymic peptides (Unicahsina) is a safe and effective therapy for the management of patients with COVID-19 respiratory onsets.

General Objective

To demonstrate the therapeutic efficacy and safety of thymic peptides (Unicahsina) in patients with COVID-19 respiratory disease.

Specific objectives

1. To evaluate the safety of thymic peptides (Unicahsina) by monitoring adverse events using the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) and side effects using the General Assessment of Side Effects (GASE).
2. Determine the time required until the participants' response, according to clinical stability parameters.
3. Determine the median number of days of hospitalization in patients diagnosed with COVID-19 and treated with thymic peptides (Unicahsina).
4. To perform survival analysis in patients diagnosed with COVID-19 and treated with thymic peptides (Unicahsina).

SECTION 2 - DESCRIPTION OF THE POPULATION

2.1 Number of participants: 20 participants.

2.2 Describe the population:

Patients with diagnosis of COVID-19 confirmed by RT-qPCR testing, viral antigen detection or antibody detection plus associated symptomatology. The study will be performed in patients over 20 years of age without distinction of sex, race, religion, or socioeconomic status. Since the population under 20 years of age presents mostly mild or asymptomatic symptoms, with a mortality rate between 0% and 0.2%, it was decided to exclude this age range.

This research will involve elderly patients and those with comorbidities. The current COVID-19 pandemic has shown that this group of patients has the highest mortality rate, exposing the need to discover safe and effective therapies. Therefore, and given the innocuous profile that thymic peptides possess, we consider their use in this population.

Should any of the participants decide to withdraw from the study voluntarily or request self-discharge, another patient will be added to the study until 20 patients have reached conclusion either by medical discharge or death within a 20-day hospitalization follow-up. Patient data will be used up to the date of voluntary withdrawal or self-discharge.

2.3 Criteria

Inclusion Criteria:

1. Confirmed case of COVID-19 by viral nucleic acid (RNA) detection, viral antigen detection, or detection of antibodies to the virus.

2. Participants that require hospitalization under Honduran Ministry of Health Guidelines for Clinical Management of COVID-19 Adult Patients: Stage IIb, defined as a patient with or without risk factors that presents with warning signs (shortness of breath, tachypnea), and altered inflammatory parameters.

3. Participants that present with at least one of the following: oxygen saturation level below 94 percent; complete blood count showing lymphopenia, neutrophilia, or both; positive C-reactive protein; chest radiography or CT scan with ground-glass opacities.

4. Male or female at least 21 years old.

Exclusion criteria

1. COVID-19 patients that do not require hospitalization under Honduran Ministry of Health Guidelines for Clinical Management of COVID-19 Adult Patients: phase IA (asymptomatic), phase IB (mild symptoms without risk factors), or phase IC (mild symptoms with risk factors).

2. Participants currently in other clinical trials evaluating experimental drugs.

3. Known history of allergic reactions to thymic peptides or calf thymus acid lysate derivatives.

4. Organ transplant recipients.

5. Women who are pregnant or breast feeding.

SECTION 3. PARTICIPANT RECRUITMENT

Patients who meet the inclusion criteria and are hospitalized in Honduran facilities designated for the care of patients with COVID-19, will be recruited. Patients over 20 years of age capable of giving their consent will be included. In case that the patient is unable to authorize participation, authorization will be requested from his legal representative.

Prior to their incorporation into the study, patients will be approached by the specialist or health personnel present on behalf of the Medical Research Group of the Catholic University of Honduras. In addition, there will be auxiliary methods with electronic devices that will allow telemedicine to be carried out between the principal investigators of the study and the potential participants. This last procedure will be carried out with encrypted methods that guarantee the confidentiality and anonymity of the patients. The purpose and procedures of the investigation will be explained, as well as clarifying any doubts that may arise. Once consent has been obtained, the clinical data registration will begin, and the application of the protocol will be coordinated with the treating physicians.

SECTION 4 - METHODOLOGY AND PROCEDURES

4.1 Study design

This is a single-arm, open-label, phase II clinical trial to evaluate the safety and efficacy of thymic peptides in the treatment of hospitalized COVID-19 patients in Honduras, at the Hospital de Santa Bárbara Integrado. A participant-level comparison will be made based on registry data of the Hospital de Santa Bárbara Integrado, after propensity score matching.

In total, 20 patients will be recruited from the designated hospital for COVID-19 patient care, including those who have signed informed consent and who meet the inclusion criteria. For the generation of the comparison group through data from clinical records, it will be carried out by authorization from the Hospital de Santa Bárbara Integrado, given its retrospective nature, without experimental intervention.

Clinical characterization

Once the protocol is started, the information corresponding to the general data, signs, symptoms, physical examination and laboratory values of each patient will be recorded daily in the data collection instrument. The record will be made on paper, which will be digitized and sent under encryption to the principal investigators every day.

As a quantitative method, the following laboratory findings will be considered to evaluate clinical progress, according to the requirement and availability of the hospital:
● Complete blood count
● C-reactive protein
● D-dimer
● Ferritin

- Procalcitonin
- Lactate
- AST
- ALT
- BUN
- Creatinine

Administration of thymic peptides (Unicahsina)

Thymic peptides (Unicahsina) will be administered in an oral dose of 250 mg per day. In the intubated patient, the administration will be done through a nasogastric tube. The presentation of thymic peptides (Unicahsina) will be in lyophilized form, which will be diluted in 50 ml of drinking water prior to administration. It should be administered on an empty stomach one hour before or two hours after a meal. If the patient is discharged, or if they need to be referred to an ICU, all the information will be gathered up to that moment and the administration of Unicahsina will be stopped.

Primary outcome measures

1) Time to participant recovery (Time Frame: During hospitalization for up to 20 days.)

Measured in days to clinical response that will be defined as clinical stability (temperature, ≤37.8°C; heart rate, ≤100 beats per min; systolic blood pressure, ≥90 mm Hg; respiratory rate, ≤24 breaths per min; oxygen saturation, ≥90 percent; normal mental status; no receipt of supplemental oxygen by face mask or mechanical ventilation) with improvement in at least one symptom (anosmia, dysgeusia, cough, shortness of breath, cyanosis, conjunctivitis, pharyngitis, sputum production, rhinorrhea, myalgia, otalgia, odynophagia, fatigue, diarrhea, hemoptysis, vomit) and with no symptom worsening that is sustained for at least 24 h.

2) Number of participants with treatment related adverse events as assessed by the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) (Time Frame: Up to 20 days)

Number of participants who experience adverse events ≥ Grade 3, as defined by the CTCAE v5.0.

3) Number of participants with treatment related side effects as assessed by the General Assessment of Side Effects (GASE) (Time Frame: Up to 20 days)

Number of participants who experience severe side effects as defined by the GASE.

Secondary Outcomes measures

1) Hospital average length of stay (ALOS) as measured by the mean participants' inpatient days (Time Frame: Up to 20 days)

The average length of stay will be calculated by dividing the sum of inpatient days by the number of participant admissions.

2) Overall survival defined as the time from the start of treatment until death due to any reason [Time Frame: Up to 20 days]

Time for overall survival will be measured in days.

Other complementary analyzes

Computerized axial tomography and chest radiography

A Computerized tomography without contrast will be performed, with the patient in the supine position and the image acquisition will be carried out at the end of inspiration. The following parameters will be requested to obtain axial images: 1.25 mm thick, 1.25 mm interval, 120 kVp. If it is possible, chest radiographs will be requested in posteroanterior projection. However, in patients whose clinical condition warrants it, anteroposterior radiography will be considered. The images will be obtained at the moment of maximum inspiration, either with instruction to the patient or by controlling the ventilator console in the case of intubated patients.

The tomographic or radiographic findings will be interpreted using the scoring system previously described by Cai et al (46). After the analysis by the assigned radiologist of the hospital center, the data generated by CT or X-ray will be submitted to two independent radiologist physicians, both blinded to the corresponding group of participants and the corresponding clinic. These specialists will assign a Pulmonary Score 1 (SP1) according to the findings they observe, based on three categories:
1 = Normal attenuation
2 = Ground-glass opacity
3 = Consolidation

Each lung will be divided into 3 zones with limits defined as follows:

Upper zone: area above the level of the carina.
Middle zone: area between the level of the carina and the inferior pulmonary vein.
Inferior zone: area below the level of the inferior pulmonary vein.

In this way, a total of 6 pulmonary areas will be delimited, each of which will receive a score according to the following scale of distribution of compromised lung parenchyma or Pulmonary Score 2 (SP2):

0 = Normal
1 = 1% to 25% involvement
2 = 25% to 50% involvement
3 = 50% to 75% involvement
4 = More than 75% involvement

The scores of each lung area will be summed to obtain the Total Affectation (AT) grade. Finally, AT will be multiplied by SP1 to obtain the Total Cumulative Scale (ETA), which comprises a range from 0 to 72. A change of "Improvement" on CT or chest X-ray will be defined as an ETA lower than the previous value obtained at the

start of treatment or from the previous imaging studies. A "worsening" change will be defined as an ETA greater than the previous value obtained at the start of treatment or previous imaging studies. The stability of the picture will be defined as "Constant" when the ETA is equal to the previous value obtained at the beginning of treatment or from previous imaging studies.

Statistical Analysis Plan

All quantitative data will be described using parameters such as the mean ± standard deviation, or as the median (minimum and maximum). Qualitative data will be described by the number of cases (proportion%). Patient characteristics will be compared using the chi-square test or Fisher's exact test for categorical data. For continuous variable data, the Wilcoxon test or Student's t test will be employed. The analysis of time to a particular event will be carried out using the Kaplan-Meier method, and the analysis of differences between groups will be calculated with the log-rank test. The Cox proportional-hazard model will be used to estimate the hazard ratio and 95% confidence interval (CI). Propensity score matching methodology will be used to generate the comparison group from the clinical records. A p-value less than 0.05 will be required to establish significance. All analyses will be performed using SPSS and GraphPad Prism software.

SECTION 5 - ANONYMITY AND CONFIDENTIALITY OF DATA

The data will be obtained from the healthcare center assigned to treat positive cases of COVID-19, through the clinical record designed as an instrument for this study. The laboratory and imaging test reports, as well as the clinical records, will be digitized and sent to the principal investigators using the SOPHOS encryption software. This last part will ensure the confidentiality of each patient's data between devices while transferred. Access will be given to the Dirección General de Vigilancia del Marco Normativo de la Secretaría de Salud de Honduras (General Directorate for Regulatory Framework Surveillance of the Ministry of Health of Honduras) for the monitoring of the study. The hospital will have a password-protected electronic device. The access will be limited exclusively to medical doctors assigned for the study.

The physical documents will be attached to the patient clinical file at the end of the hospitalization.

For the data produced by CT or chest x-ray, the HOROS software will be used to visualize and anonymized DICOM files before sending them to radiologists. The confidentiality in transferring this information will be ensured by using the SOPHOS software.

SECTION 6 - POTENTIAL RISKS AND BENEFITS

6.1 Describe potential risks and discomforts

The administration of oral thymic peptides (Unicahsina) constitutes a minimal risk intervention since its use has not reported any toxic or mutagenic effects even at high

concentrations (16). It has multiple reports on its safety and efficacy in patients with immune imbalance, pathologies such as chronic bronchitis (23-25), bronchial hyperreactivity (26), asthma (27-29), food allergy (30, 31), atopic dermatitis, immunosuppression by chemo or radiotherapy for neoplasms (32, 33), initial phases of AIDS (34-37) and thymic involution of agedness (19, 38). No additional risks are expected from the application of this treatment. However, the possibility of unknown adverse effects in patients with COVID-19 cannot be ruled out.

CT or chest x-rays carry minimal risks. Imaging procedures will not involve the administration of contrast media, so no allergic reactions or renal damage is expected. Even though exposure to CT of the thorax can increase the risk of cancer, this represents an increment of only 0.7% (47). No pregnant women will be included in this study.

The administration of thymic peptides (Unicahsina) will be orally, diluting the lyophilized product in water for ingestion. In case of impaired consciousness, difficulty swallowing, or if the medical doctors consider the necessity of intubation, a nasogastric tube will be placed for the continuation of treatment. This process carries the following risks: displacement of the tube, electrolyte dysregulation, hyperglycemia, diarrhea, constipation, vomit, tube obstruction, and pulmonary aspiration (48). However, to minimize risks, this procedure will be performed by trained healthcare personnel.

Samples for RT-qPCR will be taken using a nasal swab. This technique carries minimal risks that include pharyngeal reflex, mild nose bleeding, discomfort, and cough (49, 50).

No psychological, sociological, or legal risks are expected in this study.

6.2 Describe how risks will be minimized

Monitoring of possible adverse reactions or side effects will occur after the administration of the drug. In case reactions that compromise the health of the participant appear, the drug will be suspended. If discomfort occurs when swallowing, a way will be sought to facilitate ingestion, fractionating the doses of the drug or its volume.

In case the placement of a nasogastric tube is required for the administration of the drug, it will be placed by trained personnel.

6.3 Describe the potential benefits

The administration of thymic peptides (Unicahsina) exerts an immunomodulatory effect, which will enhance the response of T, B and Natural Killer (NK) lymphocytes (51). This response is expected to help stop or mitigate the progression of alveolar destruction induced by the inflammatory reaction in the infection by Sars-Cov-2. The objective is to prevent progression to a more severe form of the disease, and consequently to reduce the mortality rate.

SECTION 7 - INCENTIVES AND INDUCEMENTS TO PARTICIPATE

7.1 Describe any incentive/inducement to participate that will be offered to the participants.

No incentives or inducements will be offered to participate in this study.

SECTION 8 - OTHER FINANCIAL CONSIDERATIONS

8.1 Describe the monetary expenses that the participants will incur.

The participants will not incur in any monetary expenses directly related with the study. The doses of the thymic peptides (Unicahsina), as well as the RT-qPCR tests, antigen or antibody test, CT scan and chest radiographs that are required as additional controls to those requested as a protocol by the Ministry of Health of Honduras will be financed by the State of Honduras, the Universidad Católica de Honduras and the Grupo de Investigacion Medica de la Universidad Católica de Honduras (GIMUNICAH).

8.2 Describe the procedures for compensating research-related injuries

Study-related injuries are not expected. However, for the minimal risks of a slight nasal bleeding originating after sampling for RT-qPCR or some complication in the placement of a nasogastric tube in intubated patients, immediate management of the lesion will be performed, and all expenses related to this will be covered by the State of Honduras. In the event of a hypersensitivity reaction to thymic peptides (Unicahsina), timely management will be given and the necessary medication expenses for the specific event will be covered by the State of Honduras.

SECTION 9 - INFORMED CONSENT

The informed consent process will start from the recruitment of the participants. Once the potential study participants have been identified, we will proceed to report on the objective and the methodology that will be used to answer the research questions. During the discussion of the consent form, it will be ensured that the participant has the necessary time to fully understand all the interventions to which he/she will be exposed. The participants will be allowed to ask the questions that are necessary to clear any doubts. It will be emphasized that the decision to be part of the study is absolutely voluntary, in addition to the fact that participant can withdraw at any time without incurring in any penalty. If the participant agrees, he/she will be asked for a signature or fingerprint. The participant will receive a copy of the form.

During the duration of the research, the participant will continue to be informed about their clinical evolution, new risks, changes in procedures, dose modification, extension, or completion of the study (prior authorization by the Ethics Committee). In addition, the ability of the patient to consent and reaffirm their willingness to remain in the study will be continuously evaluated.

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PROTOCOL

VERSION B

SECTION 1 – TITLE, PURPOSE AND JUSTIFICATION

1.1 Title. Safety and Efficacy of Thymic Peptides in the Treatment of Hospitalized COVID-19 Patients in Honduras.

1.2 Study Purpose. To determine if thymic peptides (Unicahsina) are a new therapeutic option for the treatment of patients infected by COVID-19 in order to improve their clinical prognosis.

1.3 Justification. Coronaviruses are important pathogens in humans and animals, causing respiratory tract and lung parenchymal infections. In late 2019, a novel coronavirus was identified as the cause of a series of pneumonia cases in Wuhan, a city in Hubei province in China (1). The infection spread rapidly, leading to an epidemic throughout China, followed by an increasing number of cases in other countries, affecting all continents except Antarctica (1). WHO declared COVID-19 a pandemic on March 11, 2020 (2). The virus is currently referred to as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the disease caused by the virus is known as COVID-19.

At the time of writing this protocol, more than 5,584,091 cases of COVID-19 have been reported worldwide (3). This is largely due to the ease with which this virus is transmitted. The primary mechanism of spread is person-to-person transmission, which occurs primarily by respiratory droplets (1). Transmission of SARS-CoV-2 via fomites is plausible as it has been shown to remain viable on surfaces for hours and even several days (4).

The coronavirus that causes COVID-19 is a betacoronavirus of the same subgenus as SARS-CoV (5). The incubation period is estimated to be 14 days after exposure, with most cases occurring between 4 and 5 days (6-8). The disease is mild in 81% of cases, severe in 14% and critical in 5% (9). The overall mortality rate is 2.3% (9). The clinical course is characterized by fever (99%), fatigue (70%), dry cough (59%), anorexia (40%), myalgia (35%), dyspnea (31%) and sputum (27%) (10). Pneumonia appears to be the most serious manifestation of infection, characterized mainly by fever, cough, dyspnea and a bilateral pulmonary infiltrate (visible on imaging) (7, 10-12).

Global statistics show that mortality due to COVID-19 increases significantly with age. Pediatric patients under 9 years of age present mild cases with practically zero mortality (13). Between 9 and 39 years of age, statistics indicate a mortality of approximately 0.2% (13). In the age range between 40 and 69 years, the rate oscillates between 1.3 and 3.6% (13). Above 70 years of age, deaths due to COVID-19 increase exponentially, reaching 8% of cases (13). Patients over 80 years of age are the highest risk group with a mortality rate of 14.8% (13).

This remarkable statistical difference in morbidity and mortality is consistent with a biological process that has so far been scarcely addressed: involution of the thymus. This retrosternal gland is part of the primary lymphoid organs, playing an important role in the maturation and differentiation of T cells to CD4+ and CD8+ subtypes, in addition to secretion of cytokines that mediate the immune response (14). Involution follows a characteristic pattern in which there is a progressive loss of function from the first year of life (15). Between 35 and 45 years of age this process accelerates, losing up to 30% of its function in a decade (15). Finally, after the age of 65 it regresses and is replaced in its totality by adipose tissue (15).

Ferman-Cano et al. suggest that the relative protection observed in the infant population with COVID-19 could be explained by the influence of the thymus. Based on this background and the evident relationship between the age ranges in which thymus involution occurs and the exponential increase in mortality, we propose that at older ages there is a greater deficiency of thymic factors, whose replacement represents a therapeutic mechanism to be explored. In this context, thymic peptides stand out as potent candidates given their immunogenic properties and proven safety.

Thymomodulin is a set of low molecular weight peptides of less than 10,000 Daltons, derived from the acid lysis of calf thymus (16). It has been shown that modulates the maturation and function of T lymphocytes and favors the expression of B cell surface markers (16,17). In vitro, thymomodulin has been shown to possess a myelopoiesis-inducing effect in the bone marrow, stimulating granulocyte and macrophage colonies (18). In addition, in murine studies it has been shown to produce an increase in serum thymic hormone (19).

In clinical studies, thymomodulin has proved to reduce the number of recurrent respiratory infections in children (20). This improvement is associated with a higher CD3 + and CD4 + cell count, increased neutrophilic function and salivary IgA levels (21, 22). It also has multiple reports on its safety and efficacy in patients with immune imbalance, in pathologies such as chronic bronchitis (23-25), bronchial hyperresponsiveness (26), asthma (27-29), food allergy (30, 31), atopic dermatitis, immunosuppression by chemo or radiotherapy for neoplasms (32, 33), initial phases of AIDS (34-36) and senectitude thymic involution (19,37,38).

In geriatric patients, studies prove safety at high doses (19), an increase in the number of lymphocytes, monocytes, IgA, IgG and IgM levels (37-40). Likewise, thymomodulin increases the production of free radicals due to alveolar macrophages (23). It is noteworthy to mention that it can even prevent and emend leukopenia in patients undergoing chemotherapy and radiotherapy (41), which has been proven in patients with breast and genitourinary tract cancer (42).

In studies of acute and chronic toxicity, it has been shown that thymomodulin does not possess teratogenic or embryotoxic effects (43). For this reason, it has a high potential for use during pregnancy.

Given the fact that the population most at risk of presenting severe disease and a higher mortality rate due to COVID-19 are immunosuppressed patients, older adults and/or those with comorbidities, effective therapeutic options with high safety profiles are needed. Currently there are no treatments available that meet these

requirements for disease management. Treatment options include protocols using chloroquine or hydroxychloroquine. The evidence in favor of its use remains controversial, since a number of adverse effects have been documented that could affect the most vulnerable population. They are contraindicated in patients with retinopathy (44), neuromyopathy and heart disease due to the possible increase in the QT interval (45).

Scientific evidence proves the absence of short or long-term adverse effects of thymomodulin, obtaining significant results in the treatment of patients with immunological vulnerability in various respiratory pathologies (23). Taking this into account, it can be considered a promising alternative to combat the COVID-19 pandemic. The following sections will discuss the methodology of the clinical trial that will be used to evaluate its efficacy as a therapeutic option.

Hypothesis

Oral administration of thymic peptides (Unicahsina) is a safe and effective therapy for the management of patients with COVID-19 respiratory onsets.

General Objective

To demonstrate the therapeutic efficacy and safety of thymic peptides (Unicahsina) in patients with COVID-19 respiratory disease.

Specific objectives

1. To evaluate the safety of thymic peptides (Unicahsina) by monitoring adverse events using the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) and side effects using the General Assessment of Side Effects (GASE).
2. Determine the time to participant recovery, according to a clinical ordinal progression scale.
3. To perform survival analysis in patients diagnosed with COVID-19 and treated with thymic peptides (Unicahsina).

SECTION 2 - DESCRIPTION OF THE POPULATION

2.1 Number of participants: 20 participants.

2.2 Describe the population:

Patients with diagnosis of COVID-19 confirmed by RT-qPCR testing, viral antigen detection or antibody detection plus associated symptomatology. The study will be performed in patients over 20 years of age without distinction of sex, race, religion, or socioeconomic status. Since the population under 20 years of age presents mostly mild or asymptomatic symptoms, with a mortality rate between 0% and 0.2%, it was decided to exclude this age range.

This research will involve elderly patients and those with comorbidities. The current COVID-19 pandemic has shown that this group of patients has the highest mortality rate, exposing the need to discover safe and effective therapies. Therefore, and given the innocuous profile that thymic peptides possess, we consider their use in this population.

Should any of the participants decide to withdraw from the study voluntarily or request self-discharge, another patient will be added to the study until 20 patients have reached conclusion either by medical discharge or death within a 20-day hospitalization follow-up. Patient data will be used up to the date of voluntary withdrawal or self-discharge.

2.3 Criteria

Inclusion Criteria:

1. Confirmed case of COVID-19 by viral nucleic acid (RNA) detection, viral antigen detection, or detection of antibodies to the virus.

2. Participants that require hospitalization under Honduran Ministry of Health Guidelines for Clinical Management of COVID-19 Adult Patients: Stage IIb, defined as a patient with or without risk factors that presents with warning signs (shortness of breath, tachypnea), and altered inflammatory parameters.

3. Participants that present with at least one of the following: oxygen saturation level below 94 percent; complete blood count showing lymphopenia, neutrophilia, or both; positive C-reactive protein; chest radiography or CT scan with ground-glass opacities.

4. Male or female at least 21 years old.

Exclusion criteria

1. COVID-19 patients that do not require hospitalization under Honduran Ministry of Health Guidelines for Clinical Management of COVID-19 Adult Patients: phase IA (asymptomatic), phase IB (mild symptoms without risk factors), or phase IC (mild symptoms with risk factors).

2. Participants currently in other clinical trials evaluating experimental drugs.

3. Known history of allergic reactions to thymic peptides or calf thymus acid lysate derivatives.

4. Organ transplant recipients.

5. Women who are pregnant or breast feeding.

SECTION 3. PARTICIPANT RECRUITMENT

Patients who meet the inclusion criteria and are hospitalized in Honduran facilities designated for the care of patients with COVID-19, will be recruited. Patients over 20 years of age capable of giving their consent will be included. In case that the patient is unable to authorize participation, authorization will be requested from his legal representative.

Prior to their incorporation into the study, patients will be approached by the specialist or health personnel present on behalf of the Medical Research Group of the Catholic University of Honduras. In addition, there will be auxiliary methods with electronic devices that will allow telemedicine to be carried out between the principal investigators of the study and the potential participants. This last procedure will be carried out with encrypted methods that guarantee the confidentiality and anonymity of the patients. The purpose and procedures of the investigation will be explained, as well as clarifying any doubts that may arise. Once consent has been obtained, the clinical data registration will begin, and the application of the protocol will be coordinated with the treating physicians.

SECTION 4 - METHODOLOGY AND PROCEDURES

4.1 Study design
This is a single-arm, open-label, phase II clinical trial to evaluate the safety and efficacy of thymic peptides in the treatment of hospitalized COVID-19 patients in Honduras, at the Hospital de Santa Bárbara Integrado. A participant-level comparison will be made based on registry data of the Hospital de Santa Bárbara Integrado, after propensity score matching.

In total, 20 patients will be recruited from the designated hospital for COVID-19 patient care, including those who have signed informed consent and who meet the inclusion criteria. For the generation of the comparison group through data from clinical records, it will be carried out by authorization from the Hospital de Santa Bárbara Integrado, given its retrospective nature, without experimental intervention.

Clinical characterization

Once the protocol is started, the information corresponding to the general data, signs, symptoms, physical examination and laboratory values of each patient will be recorded daily in the data collection instrument. The record will be made on paper, which will be digitized and sent under encryption to the principal investigators every day.

As a quantitative method, the following laboratory findings will be considered to evaluate clinical progress, according to the requirement and availability of the hospital:
● Complete blood count
● C-reactive protein
● D-dimer
● Ferritin
● Procalcitonin
● Lactate
● AST

- ALT
- BUN
- Creatinine

Administration of thymic peptides (Unicahsina)

Thymic peptides (Unicahsina) will be administered in an oral dose of 250 mg per day. In the intubated patient, the administration will be done through a nasogastric tube. The presentation of thymic peptides (Unicahsina) will be in lyophilized form, which will be diluted in 50 ml of drinking water prior to administration. It should be administered on an empty stomach one hour before or two hours after a meal. If the patient is discharged, or if they need to be referred to an ICU, all the information will be gathered up to that moment and the administration of Unicahsina will be stopped.

Primary outcome measures

1) Time to participant recovery [Time Frame: During hospitalization for up to 20 days.]

Measured in days to clinical recovery that will be defined as the first day, during the 20 days after enrollment, on which a patient met the criteria for category 1, 2, or 3 on the eight-category ordinal scale. The categories are as follows: 1, not hospitalized and no limitations of activities; 2, not hospitalized, with limitation of activities, home oxygen requirement, or both; 3, hospitalized, not requiring supplemental oxygen and no longer requiring ongoing medical care; 4, hospitalized, not requiring supplemental oxygen but requiring ongoing medical care (related to Covid-19 or to other medical conditions); 5, hospitalized, requiring any supplemental oxygen; 6, hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices; 7, hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); and 8, death.

2) Number of participants with treatment related adverse events as assessed by the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5.0) [Time Frame: Up to 20 days]

Number of participants who experience adverse events ≥ Grade 3, as defined by the CTCAE v5.0.

3) Number of participants with treatment related side effects as assessed by the General Assessment of Side Effects (GASE) [Time Frame: Up to 20 days]

Number of participants who experience severe side effects as defined by the GASE.

Secondary Outcomes measures

1) Overall survival defined as the time from the start of treatment until death due to any reason [Time Frame: Up to 20 days]

Time for overall survival will be measured in days.

Other complementary analyzes

Computerized axial tomography and chest radiography

A Computerized tomography without contrast will be performed, with the patient in the supine position and the image acquisition will be carried out at the end of inspiration. The following parameters will be requested to obtain axial images: 1.25 mm thick, 1.25 mm interval, 120 kVp. If it is possible, chest radiographs will be requested in posteroanterior projection. However, in patients whose clinical condition warrants it, anteroposterior radiography will be considered. The images will be obtained at the moment of maximum inspiration, either with instruction to the patient or by controlling the ventilator console in the case of intubated patients.

The tomographic or radiographic findings will be interpreted using the scoring system previously described by Cai et al (46). After the analysis by the assigned radiologist of the hospital center, the data generated by CT or X-ray will be submitted to two independent radiologist physicians, both blinded to the corresponding group of participants and the corresponding clinic. These specialists will assign a Pulmonary Score 1 (SP1) according to the findings they observe, based on three categories:
1 = Normal attenuation
2 = Ground-glass opacity
3 = Consolidation

Each lung will be divided into 3 zones with limits defined as follows:

Upper zone: area above the level of the carina.
Middle zone: area between the level of the carina and the inferior pulmonary vein.
Inferior zone: area below the level of the inferior pulmonary vein.

In this way, a total of 6 pulmonary areas will be delimited, each of which will receive a score according to the following scale of distribution of compromised lung parenchyma or Pulmonary Score 2 (SP2):

0 = Normal
1 = 1% to 25% involvement
2 = 25% to 50% involvement
3 = 50% to 75% involvement
4 = More than 75% involvement

The scores of each lung area will be summed to obtain the Total Affectation (AT) grade. Finally, AT will be multiplied by SP1 to obtain the Total Cumulative Scale (ETA), which comprises a range from 0 to 72. A change of "Improvement" on CT or chest X-ray will be defined as an ETA lower than the previous value obtained at the start of treatment or from the previous imaging studies. A "worsening" change will be defined as an ETA greater than the previous value obtained at the start of treatment or previous imaging studies. The stability of the picture will be defined as "Constant" when the ETA is equal to the previous value obtained at the beginning of treatment or from previous imaging studies.

Statistical Analysis Plan

All quantitative data will be described using parameters such as the mean ± standard deviation, or as the median (minimum and maximum). Qualitative data will be described by the number of cases (proportion%). Patient characteristics will be compared using the chi-square test or Fisher's exact test for categorical data. For continuous variable data, the Wilcoxon test or Student's t test will be employed. The analysis of time to a particular event will be carried out using the Kaplan-Meier method, and the analysis of differences between groups will be calculated with the log-rank test. The Cox proportional-hazard model will be used to estimate the hazard ratio and 95% confidence interval (CI). Propensity score matching methodology will be used to generate the comparison group from the clinical records. A p-value less than 0.05 will be required to establish significance. All analyses will be performed using SPSS and GraphPad Prism software.

SECTION 5 - ANONYMITY AND CONFIDENTIALITY OF DATA

The data will be obtained from the healthcare center assigned to treat positive cases of COVID-19, through the clinical record designed as an instrument for this study. The laboratory and imaging test reports, as well as the clinical records, will be digitized and sent to the principal investigators using the SOPHOS encryption software. This last part will ensure the confidentiality of each patient's data between devices while transferred. Access will be given to the Dirección General de Vigilancia del Marco Normativo de la Secretaría de Salud de Honduras (General Directorate for Regulatory Framework Surveillance of the Ministry of Health of Honduras) for the monitoring of the study. The hospital will have a password-protected electronic device. The access will be limited exclusively to medical doctors assigned for the study.

The physical documents will be attached to the patient clinical file at the end of the hospitalization.

For the data produced by CT or chest x-ray, the HOROS software will be used to visualize and anonymized DICOM files before sending them to radiologists. The confidentiality in transferring this information will be ensured by using the SOPHOS software.

SECTION 6 - POTENTIAL RISKS AND BENEFITS

6.1 Describe potential risks and discomforts

The administration of oral thymic peptides (Unicahsina) constitutes a minimal risk intervention since its use has not reported any toxic or mutagenic effects even at high concentrations (16). It has multiple reports on its safety and efficacy in patients with immune imbalance, pathologies such as chronic bronchitis (23-25), bronchial hyperreactivity (26), asthma (27-29), food allergy (30, 31), atopic dermatitis, immunosuppression by chemo or radiotherapy for neoplasms (32, 33), initial phases of AIDS (34-37) and thymic involution of agedness (19, 38). No additional risks are

expected from the application of this treatment. However, the possibility of unknown adverse effects in patients with COVID-19 cannot be ruled out.

CT or chest x-rays carry minimal risks. Imaging procedures will not involve the administration of contrast media, so no allergic reactions or renal damage is expected. Even though exposure to CT of the thorax can increase the risk of cancer, this represents an increment of only 0.7% (47). No pregnant women will be included in this study.

The administration of thymic peptides (Unicahsina) will be orally, diluting the lyophilized product in water for ingestion. In case of impaired consciousness, difficulty swallowing, or if the medical doctors consider the necessity of intubation, a nasogastric tube will be placed for the continuation of treatment. This process carries the following risks: displacement of the tube, electrolyte dysregulation, hyperglycemia, diarrhea, constipation, vomit, tube obstruction, and pulmonary aspiration (48). However, to minimize risks, this procedure will be performed by trained healthcare personnel.

Samples for RT-qPCR will be taken using a nasal swab. This technique carries minimal risks that include pharyngeal reflex, mild nose bleeding, discomfort, and cough (49, 50).

No psychological, sociological, or legal risks are expected in this study.

6.2 Describe how risks will be minimized

Monitoring of possible adverse reactions or side effects will occur after the administration of the drug. In case reactions that compromise the health of the participant appear, the drug will be suspended. If discomfort occurs when swallowing, a way will be sought to facilitate ingestion, fractionating the doses of the drug or its volume.

In case the placement of a nasogastric tube is required for the administration of the drug, it will be placed by trained personnel.

6.3 Describe the potential benefits

The administration of thymic peptides (Unicahsina) exerts an immunomodulatory effect, which will enhance the response of T, B and Natural Killer (NK) lymphocytes (51). This response is expected to help stop or mitigate the progression of alveolar destruction induced by the inflammatory reaction in the infection by Sars-Cov-2. The objective is to prevent progression to a more severe form of the disease, and consequently to reduce the mortality rate.

SECTION 7 - INCENTIVES AND INDUCEMENTS TO PARTICIPATE

7.1 Describe any incentive/inducement to participate that will be offered to the participants.

No incentives or inducements will be offered to participate in this study.

SECTION 8 - OTHER FINANCIAL CONSIDERATIONS

8.1 Describe the monetary expenses that the participants will incur.

The participants will not incur in any monetary expenses directly related with the study. The doses of the thymic peptides (Unicahsina), as well as the RT-qPCR tests, antigen or antibody test, CT scan and chest radiographs that are required as additional controls to those requested as a protocol by the Ministry of Health of Honduras will be financed by the State of Honduras, the Universidad Católica de Honduras and the Grupo de Investigacion Medica de la Universidad Católica de Honduras (GIMUNICAH).

8.2 Describe the procedures for compensating research-related injuries

Study-related injuries are not expected. However, for the minimal risks of a slight nasal bleeding originating after sampling for RT-qPCR or some complication in the placement of a nasogastric tube in intubated patients, immediate management of the lesion will be performed, and all expenses related to this will be covered by the State of Honduras. In the event of a hypersensitivity reaction to thymic peptides (Unicahsina), timely management will be given and the necessary medication expenses for the specific event will be covered by the State of Honduras.

SECTION 9 - INFORMED CONSENT

The informed consent process will start from the recruitment of the participants. Once the potential study participants have been identified, we will proceed to report on the objective and the methodology that will be used to answer the research questions. During the discussion of the consent form, it will be ensured that the participant has the necessary time to fully understand all the interventions to which he/she will be exposed. The participants will be allowed to ask the questions that are necessary to clear any doubts. It will be emphasized that the decision to be part of the study is absolutely voluntary, in addition to the fact that participant can withdraw at any time without incurring in any penalty. If the participant agrees, he/she will be asked for a signature or fingerprint. The participant will receive a copy of the form.

During the duration of the research, the participant will continue to be informed about their clinical evolution, new risks, changes in procedures, dose modification, extension, or completion of the study (prior authorization by the Ethics Committee). In addition, the ability of the patient to consent and reaffirm their willingness to remain in the study will be continuously evaluated.

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SUMMARY OF CHANGES

Change	Reason
Original: Time to Participant Response [Time Frame: During hospitalization for up to 20 days.] Measured in days to clinical response that will be defined as clinical stability (temperature, ≤37.8°C; heart rate, ≤100 beats per min; systolic blood pressure, ≥90 mm Hg; respiratory rate, ≤24 breaths per min; oxygen saturation, ≥90 percent; normal mental status; no receipt of supplemental oxygen by face mask or mechanical ventilation) with improvement in at least one symptom (anosmia, dysgeusia, cough, shortness of breath, cyanosis, conjunctivitis, pharyngitis, sputum production, rhinorrhea, myalgia, otalgia, odynophagia, fatigue, diarrhea, hemoptysis, vomit) and with no symptom worsening that is sustained for at least 24 h. **Final:** Time to Participant Recovery [Time Frame: During hospitalization for up to 20 days.] Measured in days to clinical recovery that will be defined as the first day, during the 20 days after enrollment, on which a patient met the criteria for category 1, 2, or 3 on the eight-category ordinal scale. The categories are as follows: 1, not hospitalized and no limitations of activities; 2, not hospitalized, with limitation of activities, home oxygen requirement, or both; 3, hospitalized, not requiring supplemental oxygen and no longer requiring ongoing medical care; 4, hospitalized, not requiring supplemental oxygen but requiring ongoing medical care (related to Covid-19 or to other medical conditions); 5, hospitalized, requiring any supplemental oxygen; 6, hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices; 7, hospitalized, receiving invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); and 8, death.	The time to recovery assessment was modified from the first protocol version to better adhere to World Health Organization (WHO) recommendations on clinical progression outcomes.

Original: Hospital Average Length of Stay (ALOS) as Measured by the Mean Participants' Inpatient Days [Time Frame: Up to 20 days] The average length of stay will be calculated by dividing the sum of inpatient days by the number of participant admissions. **Final:** Only overall survival as secondary outcome	Hospital average length of stay was removed as a secondary outcome in the last version of the protocol for considering it a less reliable metric. However, it was analyzed as a complementary analysis using the Kaplan-Meier method.