Research Article

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Research study to evaluate the clinical effectiveness of the nutritional supplement VITAMIC BIOSEN®, combination of Curcumin, Vitamin C and Boswellia Serrata on individuals with symptoms consistent with LONG COVID who have been vaccinated against the SARS-CoV2

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ABSTRACT

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Background and Context

As the number of patients with LONG COVID increases, so does the experience in the management of patients with COVID-19 and the knowledge surrounding its follow-up and evolution. In these circumstances, patients with persistent symptoms (lasting more than 4 weeks from the onset of symptoms) have become evident. This condition has been termed post-acute COVID syndrome (PERSISTENT COVID) or LONG COVID [1-7]. Some studies estimate that LONG COVID affects 10% of patients with COVID-19. The likelihood of developing this disease does not seem to be related to the severity in the acute phase [5], i.e., some risk factors associated with a poor prognosis in the acute phase (male sex, older age, or comorbidities) do not seem to be factors substantially related to the development of LONG COVID. In fact, current data suggest that patients with LONG COVID are predominantly female (78.9%), aged 30-59 years (86.9%), and only about 8% had been previously admitted to hospital [8]. About 65% of patients with LONG COVID may have symptoms for more than 6 months [8].

Post-acute COVID syndrome is a multi-organ disease. The predominant symptoms are dyspnea, cough, asthenia, anosmia, ageusia, headache and mental confusion [8-10]. The clinical presentation may be in the form of attacks, or in a more stable continuous form. Significant deficits in activities of daily living, including self-care and social activities, have been frequently reported. Although much progress has been made in understanding the underlying causes of SARS-CoV-2 in the acute phase, there is much unknown in LONG COVID [4,6]. It is unclear whether chronic symptoms are due to the consequences of SARS-CoV-2 infection in multiple organs, or to indirect effects such as hyperactivation of the immune system and the development of autoimmunity. The cytokine-mediated inflammatory response is central in LONG COVID. There are publications

characterizing a differentiated response. Markers of this immune-mediated response in LONG COVID are interleukin 6 (IL-6), vascular endothelial growth factor (VEGF) and interferon gamma (IFNG) [11].

The treatment of patients, suffering from LONG COVID, has been rather disappointing so far. Fortunately, with the support of VITAMIC BIOSEN® there is now a nutritional supplement which, as a supplement to the treatment prescribed by the physician, provides a promising effect in improving the symptomatology associated with many of these patients. A key breakthrough has been achieved by combining proven therapeutic compounds with the award-winning MyCell technology. The active compounds in VITAMIC BIOSEN® are Boswellia serrata, Curcuma longa and Vitamin C. While these original compounds have a very low bioavailability and therefore a very low efficacy, the MyCell technology can overcome these problems. It shows a reduced first-pass effect, almost 100% bioavailability and, most importantly, a greatly improved ability to penetrate target cells.

Boswellia serrata extract shows multiple beneficial activities in the treatment of similar symptoms experienced by COVID-19 patients. The promising effect of Boswellia can be attributed to its antioxidant, anti-inflammatory, immunomodulatory, cardioprotective, anti-platelet aggregation, antibacterial, anti-fungal, and broadly antiviral activity. Boswellia acts through multiple mechanisms. The most common mechanism may be through direct interaction with interleukin-b kinases and inhibition of gene expression. However, the most recently proposed mechanism involves Boswellia not only inhibiting the formation of classical 5-lipoxygenase products, but also producing anti-inflammatory modulators. In conclusion, Boswellia serrata extract may be useful in improving the adaptive immune response in mild to moderate symptoms of COVID-19. However, Boswellia may be beneficial in suppressing uncontrolled activation of the innate immune response [12]. It has a retroviral effect against herpes virus due to inhibition of NF-κB, essential for virus replication, and a significant reduction in NF-κB and p38 MAP-kinase activation, with reduced expression of TNF-α (tumour necrosis) and IL-1β and IL-6 [13] (Figures 1-4).

Curcumin, the active ingredient in curcuma longa, exhibits anti-inflammatory, antioxidant, antimicrobial, hypoglycaemic, wound healing, chemopreventive, chemosensitizing and radiosensitizing properties [14]. Several curcumin derivatives have been shown to have antiviral properties. The plant Curcuma longa has been used for centuries with a good safety profile. It has also been shown to be effective against influenza A viral infections by regulating the immune response to prevent lung tissue injury [14,15]. Vitamin C: Fatigue is common in patients following viral and other infections. Effective treatment options are still very scarce. The feasibility of vitamin C in post-viral fatigue, especially in acute COVID syndrome, was reassessed through a systematic review of the literature. A significant reduction in fatigue scores was observed in the vitamin C group compared to the control. Symptoms accompanying fatigue, such as sleep disturbances, lack of concentration, depression, and pain, were also frequently alleviated. Oxidative stress, inflammation, and circulatory disorders, which are important contributors to fatigue, are also addressed in prolonged fatigue due to COVID. Therefore, the antioxidant, anti-inflammatory, and endothelial restorative and immunomodulatory effects of intravenous vitamin C could be a suitable treatment option [16].

Another aspect/variable to consider is that persistent COVID occurs in patients with negative microbiological tests, but with residual, persistent or recurrent symptoms, such as fatigue, shortness of breath, cough, myalgia, arthralgia, sleep disturbances, headache, cognitive impairment, and skin rashes. Symptoms last for more than 12 weeks and cannot be attributed to an alternative diagnosis, although laboratory and radio pathological findings suggest that LONG COVID partly simulates an immune-mediated chronic inflammatory (rheumatic) disease. In fact, numerous patients have autoantibodies suggestive of a post-COVID-19 autoimmune reaction. Likewise, MRI has revealed changes in the main target organs of the SARS-CoV-2 coronavirus in these patients that correlate with inflammatory markers, again suggesting a link between organ damage and immune-inflammatory dysfunction in patients with LONG COVID. Numerous phenotypic features associated with LONG COVID have been described, and a meta-analysis of the clinical phenotype of patients with LONG COVID has recently been published.

The identification of molecular biomarkers to help us understand the pathogenic mechanisms and the heterogeneous pathophysiological response that the infection triggers in the main target organs of SARS-CoV-2 is urgently needed. However, the lack of appropriate diagnostic technologies has hindered the implementation of Precision Medicine to contribute to better management of patients with COVID-19. Therefore, progress in this direction could help us to better classify COVID-19 patients, differentiating them into pathogenic and molecular pathophysiological subtypes, which in turn would suggest new forms of clinical and therapeutic management at each stage of the disease, from outpatient control to ICU stay. The functional brain-hepatopulmonary axis is a preferred target of COVID19 and its pathophysiological response plays a crucial role in the clinical severity of the disease, but also in its long-term sequelae [17,18]. After almost two years of pandemic, and with an alarming increase in cases with LONG COVID syndrome, the study of molecular biomarkers of COVID-19 is now urgently needed. The results of this study are based on the results of the encephalo-hepato-pulmonary axis studies that help to objectify the pathogenic mechanisms and the pathophysiological response that the infection has triggered in the brain, liver, and lung, often already altered by previous pathologies

of the infected patient, including metabolic syndrome, as shown in the attached scheme.

Design

Hypothesis

The hypothesis to be tested in this study is that the use of VITAMIC BIOSEN®, in a dose of 10 drops twice a day orally for 6 weeks added to the possible treatments the patient is taking, improves the functional state and symptomatology of patients suffering from post-acute COVID syndrome (LONG COVID). In addition, and on this basis, the project aims to study the encephalo-hepato-pulmonary immuno-inflammatory reactivity, bringing together a set of organ-specific molecular biomarkers that will help us to understand the pathogenic and physio pathological molecular patterns of the patient.

Inclusion Criteria

Selection criteria and participant management

- Screening of patients was based on subjects attending consultations in medical practices and on associations caring for patients suffering from LONG COVID. Subjects who met the overall inclusion criteria were invited to participate in the study.

Condition Under Study:

- Persisting post-acute COVID syndrome (PACS) symptomatology of more than one month, with Post Covid Functional Score (PCFS) between one and four.
- Individuals between 18 and 80 years of age presenting some of the following symptoms: cough, dyspnea, asthenia anosmia, ageusia, headache and mental confusion developed in the introduction and context.
- The patient must have received at least one dose of any vaccine offered by the Spanish health system.
- The patient agrees to take the VITAMIC BIOSEN® food supplement, assigned during the 6 weeks.
- The patient expressly accepts to participate in the study through the Electronic Data Collection Notebook, eCRD. (EXPRESS ACCEPTANCE. APPENDIX I) and, if he/she is part of cohort B, he/she will also sign the informed consent form (APPENDIX II) after having been informed about his/her participation.

Exclusion Criteria

- Known hypersensitivity to VITAMIC BIOSEN®.

Active Malignancy

- Current or recent chemotherapy treatment (<6 months)
- Medical history of Human Immunodeficiency Virus (HIV) infection, or any serious
- immunocompromised state
- Use of montelukast or zafirlukast ≤ 30 days prior inclusion
- Having participated in another clinical trial in the previous month
- Women that are pregnant or breastfeeding.

Subject Withdrawal Criteria

Patients were able to voluntarily withdraw their consent to participate in the study for any reason and at any time, without providing any explanation. However, the investigator would ask about the reason and recorded the information, provided the patient had no concerns supplying relevant information freely. Study treatment was then continued, and no further evaluations were done. No further attempts to contact the patient were allowed or performed, unless there were safety results that required communication or follow-up. Subjects who voluntarily withdrew from the study were not replaced. Study data, obtained from patients that withdrew their participation, has been included in the analysis, except any data where patients contradicted at withdrawal.

Loss During the Clinical Follow-Up

In the case of patients whose current status was unknown because they did not show up (or respond by telephone) to study visits, without declaring their intention to withdraw, the investigator attempted to contact the patient, or their physician (a minimum of 3 phone calls across different days) and annotated in the original document the steps taken to contact the patient, for example, dates of phone calls. It was not considered a loss of the follow-up of the clinical trial until the last visit had passed.

Main Objective

To evaluate that the product VITAMIC BIOSEN® improves the functional status and symptomatology of the patients, by means of the changes observed in the score of the scales used in each patient after six weeks of follow-up.

Secondary Objective

To evaluate the relationship between changes in the symptomatology of individuals and changes in specific biomarkers of immuno-inflammation on the encephalo-hepato-pulmonary axis.

Research Methods

Research Design

A low-intervention, single-arm, open-label study with 60 patients suffering from post-acute COVID syndrome (LONG COVID). These patients comprise Cohort A, the entire study

population. The patients will additionally ingest 10 drops of the food supplement VITAMIC BIOSEN® twice daily. VITAMIC BIOSEN® is an approved food supplement with extensive experience of use and an excellent safety profile. As the basic type of treatment in these patients with LONG COVID remains unchanged, this study is considered to have a very low level of intervention.

For the Primary Endpoint Cohort A:

Individual self-completed surveys will be conducted by all study participants via the eCRD (Electronic Data Collection Booklet ANNEX III). These will be carried out on the day of inclusion in the study, before starting treatment, and in the sixth week at the end of treatment.

Two scales will be completed:

- Post-COVID Functional Scale (PCFS) (ANNEX IV) and assessing post-COVID symptomatology.
- EuroQol5D Quality of Life Questionnaire (ANNEX V). Screenshot of the eCRD that collects it is shown (Figure 1).

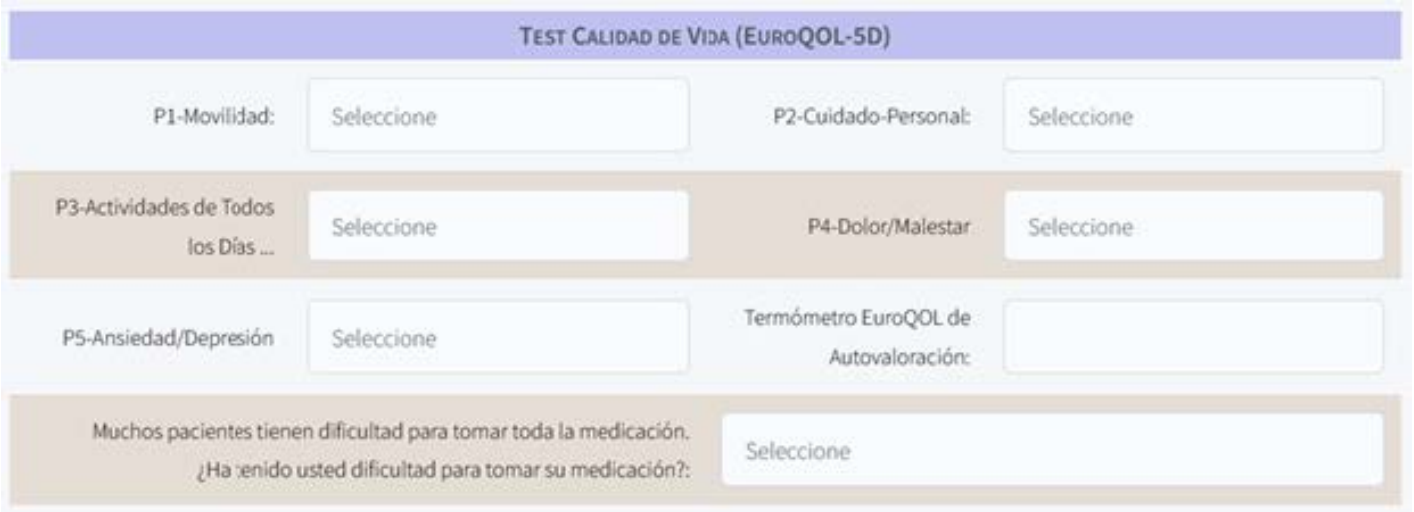

Figure 1.

After completing the surveys on the day of inclusion, they will start taking the vitamins, VITAMIC BIOSEN®, at a dose of 10 drops twice a day orally for 6 weeks in addition to any treatments the patient may be taking under normal clinical practice. All data will be collected in the eCRD created for the project, which will be stored encrypted and after the use of the corresponding pseudo-anonymisation procedure for the protection of personal data.

For the Secondary Objective, Cohort B:

A subgroup of 20 participants (making up Cohort B) will have blood tests performed in routine clinical practice. A sample of venous peripheral blood (3-4 ml) will be taken from the arm, fasting and first thing in the morning. After 6 weeks, the indicated test will be repeated, and results will be compared between the baseline and final situation. The study will use the multi-analytical molecular laboratory test ENCHEPAX®, from the company PERSONA BIOMED SPAIN SL (ANNEX VI). All data will be collected in the eCRD created for the project, which will be stored encrypted and after the use of the corresponding pseudo-anonymisation procedure for the protection of personal data.

Selection, Location ADN Follow Up, of Participants

The participants were selected after an open process of communication of the study through Spanish Associations of patients with LONG COVID and Primary Care consultations in different parts of the country.

Inclusion in the study was carried out after a personal interview with each of the candidates, in which it was verified that they met all the inclusion criteria and none of the exclusion criteria, while they were informed of the study (Objectives, Methodology, Product Characteristics, etc.), as well as the need to sign a personal consent form for participation.

Finally, 60 patients were included in the study and were assigned to the two cohorts as follows:

- Cohort A (does not include biomarker analysis): made up of 40 patients who met all the criteria and who, for various reasons (place of residence, self-will, reduced mobility,...), did not wish to undergo blood collection twice (as indicated in the protocol), to determine biomarker values.

- Cohort B (includes biomarker analysis), formed by 20 patients, who communicated their willingness and disposition to undergo both extractions in the clinics authorized for this purpose and on the dates indicated in order to comply with the deadlines established in the protocol. Likewise, in order to create a homogeneous cohort, the persons with the most similar clinical, anthropometric and hygienic and dietary profile were selected.

The individuals were selected from all the Spanish territory and the follow-up was carried out by means of personal interviews with the research team of the study.

Likewise, an e-mail address was set up on the web site created for the study to attend to any doubts or questions that the participants might have during the 6 weeks of follow-up of the study. In this regard, 19 queries were registered through this system, which were answered by the principal investigator.

For the extraction of the blood samples necessary for the biomarker test, 4 clinics with clinical analysis services were selected, located in the cities of:

MADRID, BARCELONA, CASTELLON AND VALENCIA.

Each patient underwent two extractions in the same center 6 weeks apart and after treatment with VITAMIC BIOSEN.

The samples obtained were processed according to the procedures defined by the manufacturer of the ENCHEPAX™ test, and were duly coded to ensure compliance with the data protection law.

The analytical procedure was performed at the facilities of PERSONA BIOMED SPAIN, as the manufacturer of the ENCHEPAX test, and all samples were processed by them on the same analytical plate to avoid any possible distortion factor.

Evaluation of the Response

Primary outcomes Functional status, according to Post-COVID Functional Scale (PCFS) one, two, three and six weeks after treatment initiation. EuroQol5D Quality of Life Questionnaire. Secondary outcomes Symptomatology according to the 10-point Likert scale one, two, three and 6 weeks after treatment initiation. The following symptoms were measured:

- Dyspnea
- Cough
- Asthenia
- Anosmia
- Ageusia
- Headache
- Mental confusion

Selection criteria and participant management Screening of patients was based on subjects attending consultations in medical practices and on associations caring for patients suffering from LONG COVID. Subjects who met the overall inclusion criteria were invited to participate in the study.

Description of Individual Interviews

The collection of data was done by in-person interviews (mandatory at first assessment) and by in-person interviews or phone calls after one, two, three or six weeks. Day one (1) was the day of first intake. On the day of inclusion and first assessment, the investigator carefully explained the aim and the design of the study and delivered a relevant information sheet. Following this, the patient signed the consent form. Data was collected by healthcare professionals, either nurses or medics. Following the initial assessment, a decision was made for inclusion of the candidate, given all inclusion criteria work and no exclusion criteria harm. Following this, two jars of Vitamic Biosen were provided to the patient and a demonstration was done to show how the portioning by drops should be done.

Case Report Form (CRF)

A database tool was designed specifically for this study. The data was registered at the moment of the interview, either on paper, or electronically. Concommittant medication was documented. Investigators were encouraged to just observe and document what they saw and heard in a highly objective way. Key tools that were used were the Post Covid Functional Scale (PCFS), EuroQol5D Quality of Life Questionnaire and 10-point Likert Scales regarding Dyspnea, Cough, Asthenia, Anosmia, Ageusia, Headache and Mental Confusion.

Main Objective

The changes in the symptomatology collected between the responses completed at baseline and the responses completed at six weeks for the two scales used will be studied.

Secondary Objective

Definition of clinical-biological phenotypes and endotypes. This will be done by identifying phenotypes and endotypes based on the patterns defined by plasma levels and interrelationships of brain-hepatopulmonary molecular biomarkers and their clinical-biological correlations. On this basis, patients will be stratified and their differential diagnosis with other pathologies, their prognosis and level of follow-up and the therapeutic opportunities that will benefit them most will be identified in each subgroup of patients. Phenotypes will be a manifestation of the interaction between the genetics of the individual and their environment. They may change over time and will be defined according to observable characteristics, be they clinical, morpho pathological, biochemical and physiological, or response to treatment, even if unrelated to the underlying pathophysiology. As a result, phenotypes will overlap with each other, and classification will be difficult.

In contrast, endotypes will represent specific patterns of biological expression and will be associated with pathogenic mechanisms of disease, or pathophysiological mechanisms of response to disease, and will explain the observable properties of a phenotype. The results of this project will allow the discussion of new perspectives that may arise from the molecular classification of LONG COVID into phenotypes and endotypes, with special emphasis on the usefulness of this molecular re-classification in personalising patient therapeutics. An endotype is recognised ''*where a specific biological pathway is identified that explains the observable properties of a phenotype*''.

Practical Aspects

Description of Individual Self-Interviews

Data collection will be done by means of individual self-completed surveys in a way that facilitates completion by participants (face-to-face surveys are mandatory for those included in the biomarker group, cohort B) at the start of the study and again after six weeks. The first day (1) is the day of first intake. On the day of inclusion and the first assessment, the objective and design of the study will be carefully explained and it will be mandatory that the patient accepts the express informed consent in the eCRD, this express consent generates an automatic record of compliance and if not signed, the patient will not be able to continue with the questionnaires and will be automatically excluded from the study. In order to collect the self-interviews, an Electronic Data Collection Booklet «eCRD» has been generated. The data collection process for the study is carried out using a web page created ad hoc for this purpose. The person who will participate in the study will register beforehand, filling in the Participant Profile form, where he/she will indicate the minimum data to know if he/she meets the inclusion criteria and none of the exclusion criteria. The registration process is carried out by indicating a personal (non-corporate) e-mail address, after which the system sends them access to this e-mail address, thus guaranteeing that they have access to it. This access gives them access to define a password that is securely stored in the system. From that moment on, the user can access the web platform, using the e-mail address and passwords provided by the user. Once identified, the system takes them to the Participant Profile form, where they are obliged to fill in the data necessary for the study to be carried out. The system will take them to this form again and again until they have answered all the questions it contains. And above all, it includes their express acceptance of their participation and the transfer of data for the study.

Once the registration has been completed, the selection process is carried out according to the inclusion and exclusion criteria of the study. If they meet the inclusion criteria, they will be informed and will then be able to continue with the study. From the moment the participant has been selected, on entering the platform and after identifying themselves, they will have access to the study follow-up questionnaires. The first access will take them to a form where they will fill in the Post COVID-19 Functional Status Scale (PCFS) and the EuroQOL-5D Quality of Life Scale, which will reflect their general state at the start of the study. Once you have completed both, you will then be able to access a new form where you will indicate to which address we have to send the package containing the vitamins for immediate delivery. During the duration of the study, 6 weeks from the start of taking the supplements, you will be able to access the platform as many times as you want, but you will not be able to access the final questionnaires of the study until these 6 weeks have elapsed. What will be available to you during this time is the ability to opt-out and cancel follow-up in the study at any time, and although we will ask for an explanation of the opt-out/cancellation, it is completely optional. Once 6 weeks have elapsed since the start of the study, access will be given to new forms with the same previous scales, thus collecting the values for comparison with those previously supplied. Their participation in the study will be closed with a satisfaction form and the issue of a certificate of participation in this study of a personal nature.

In the case of patients included in the biomarker group, they will have both interviews, first in person, where they will be given and explained the relevant information sheet. After this information, the patient will sign the consent form. The information sheet and consent form are attached (ANNEX II). The data will be collected by healthcare professionals, either nurses or doctors. Only doctors will be able to sign the relevant documents.

Statistical Analysis

The statistical analysis will be descriptive. The change in the three scales will be described, comparing the scales on the day of inclusion with those at week six. A description will also be given

for each of the seven 10-point Likert scales, comparing the item values on the day of inclusion with those at week six. Convenience sampling, which is a non-probability, non-random sampling technique, will be carried out in which the persons included in the research project are purposively determined on the basis of the inclusion and exclusion criteria mentioned above. Subsequently, the type of variables collected in the study will be determined as a preliminary step in order to choose the most appropriate and valid methods of analysis of the dependent variables, independent variables, factors related to the patient, the disease and the treatment, which will be studied to determine whether or not there is an association with the dependent variable. Measures of central tendency (mean, median and mode) and dispersion will be used for quantitative variables and, in the case of qualitative variables, data will be collected in frequency tables, expressed in terms of proportion and noted in brackets. In order to carry out association studies, a univariate analysis will be carried out for each of the independent variables mentioned above.

To compare groups of categorical variables, the chi-square (X2) and Fisher's exact probability tests will be used as hypothesis tests; the Student's t-test or the Mann-Whitney U-test will be applied for quantitative data. To compare continuous quantitative variables in related samples, the t-test shall be used if the distribution is normal or the Wilcoxon signed-rank test if the distribution is non-normal. The significance level will be considered at < 0.05 (two-tailed). Hierarchical cluster analysis will be used to group patients according to their pattern of similarity by plasma levels of the biomarkers studied. The analysis will use mathematical algorithms to quantify the similarities between subjects within each subgroup and define subgroups where similarities are strongest between patients in the same subgroup and weakest in relation to patients in other subgroups. Hierarchical cluster analysis will also identify patterns of association between molecular and genetic biomarkers, which will help to interpret mechanistic relationships in the processes in which they are involved. Multivariate analyses such as principal component analysis will be applied to identify patient subgroups and the biomarkers that best define them. Correlations between canonical variables and derived algorithms will be used to integrate clinical and analytical variables in the classification of patients with respect to their level of response to the different therapeutics applied. The statistical package SPSS version 12.0 will be used.

Research Schedule (Tentative)

Key Dates

- Submission of documentation to the ethics committee: May 2022.
- Recruitment: May-June 2022.
- Completion of testing: July-August 2022.
- Analysis of results: September 2022
- Draft report
- Draft final study report: October 2022.

Ethical Considerations

The study will be governed by the basic ethical principles contained in the Declaration of Helsinki (WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI. Ethical Principles For Medical Research Involving Human Subjects. Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1983; 41st WMA General Assembly, Hong Kong, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 and note of clarification on paragraph 29 added by the WMA General Assembly, Washington 2002) and in the Spanish regulations in force for the performance of observational studies (SAS/3470/2009, of 16 December) and will be subject to prior approval by the Ethics Committee of any of the hospitals involved.

Data Confidentiality

The highest standards of professional conduct and confidentiality will always be maintained and the current national legislation on data protection (Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales (LOPD) y garantía de los derechos digitales) and the Ley 14/2007, de 3 de julio, de Investigación Biomédica (Law 14/2007, of 3 July, on Biomedical Research) will be complied with. The study complies with the provisions of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data. Protection of personal data: Confidentiality and anonymity will be supported according to the Organic Law 3/2018 of 5 December on the protection of personal data and guarantee of digital rights and the EU Regulation 2016/679 on the protection of personal data and the Council of 27 April 2016 on Data Protection (GDPR) concerning personal data and the free movement of such data of the European Parliament and the Council of the European Union, and following the recommendations of the Declaration of Helsinki (Ethical Principles for Human Medicine Research 2013), both for the implementation phase and for the presentations and publications derived from the study. In the eCRD, subjects will be identified only with a code. No other information regarding the possible identification of participants will be included. The name of the participants will not appear in any publication or communication of the results of the study.

Information and Informed Consent Sheet Cohort B. Annex ii

Participants will be asked to expressly agree to participate, respecting their autonomy of decision. All participants will have to accept an express consent in the eCRD in order to be included (ANNEX I) and in addition an informed consent (Information and Informed Consent Form). Information and informed consent. ANNEX II) explained by the healthcare professional before starting their inclusion in the study and the subsequent blood collection for the secondary endpoint. The investigator will proceed to explain the study and will read the information sheet in front of the patient, clarifying any doubts that may arise during the reading.

The investigator shall ascertain whether the patient has understood everything clearly and if the patient agrees to participate in the clinical study, the patient's signature for informed consent shall be collected. Consent shall be documented by the patient's signature and date on the appropriate form, together with the date and signature of the person responsible for the submission of the study and the request for consent.

Patients or their legal representatives should be informed in a timely manner of any information that may have a relevant influence on the patient's willingness to continue participation in the study. Communication of this information shall be made and documented by means of a revised consent form, or an addendum to the original consent form. Two copies of the informed consent shall be signed. One copy shall be retained by the participant and one copy shall be retained by the investigator and kept in the investigator's file along with the other study documents. In addition, it shall be documented in the ECRD that the participant agrees to participate in the clinical trial and signs the informed consent.

Results

In general, we observe a significant downward trend. Dyspnea, sleep disorders, headache and depression significantly improved after giving VITAMIC BIOSEN®, pain, mobility intestinal problems and concentration problems were slightly improved.

- Dyspnea: a significant difference was seen in reported symptom intensity
- Sleep disorders: a significant difference was seen in reported symptom intensity
- Headache: a significant difference was seen in reported symptom intensity in each visit.
- Depression: a significant difference was seen in reported symptom intensity in each visit.
- Pain: a significant difference was seen in reported symptom intensity in each visit.
- Mobility intestinal problem: a significant difference was seen in reported symptom intensity in each visit.
- Mental confusion: a significant difference was seen in reported symptom intensity

For dyspnea, sleep disorders, headache and depression, pain, mobility intestinal problems and concentration problems one or more p-values have been less than 0.05, meaning that there are significant differences between the mean severity reported at each visit for each symptom. It also indicates that there are significant differences in the severity of symptoms collected between visits when compared two by two.

Population Data

A total of 60 patients are being monitored at this primary care clinic. The number of visits and the number of patients per visit are shown on the table below.

Demographics

90% of patients are woman with a mean age of 42.55 years old.

Symptoms

Mental confusion: 69. 38 %

Headache: 77.5 %

Dyspnea: 69.38

Mobility intestinal problem: 58.9%

Asthenia: 79.58%

Pain: 22.45%

Sleep disorder: 34%

Vaccines

100 % of patients were already vaccinated at first interview.

Results

In general, we observe a significant downward trend in all symptoms except Anosmia and Ageusia.

- Dyspnea: a significant difference was seen in reported symptom intensity.
- Asthenia: a significant difference was seen in reported symptom intensity (visit 3, 4 and 5).
- Headache: a significant difference was seen in reported symptom intensity (visit 3, 4 and 5).
- Mental confusion: a significant difference was seen in reported symptom intensity (visit 5).
- Pain a significant difference was seen in reported symptom intensity

- Mobility intestinal: a significant difference was seen in reported symptom intensity
- Sleep disorder a significant difference was seen in reported symptom intensity
- Depression: a significant difference was seen in reported symptom intensity

The following tables show the changes in the biological markers of damage to the encephalo-hepato-pulmonary axis.

- A reduction in inflammation was observed with a marked decrease in CPR, as well as a reduction in markers of enterohepatic involvement.
- Increase in liver acute phase reactant proteins (AGP, A2M, CRP).
- Increased levels of these three acute phase reactant proteins suggest subclinical coronavirus infection subclinical coronavirus infection in the GI tract, with mucosal permeability disorder and possible dysbiosis.
- It may lead to a comprehensive disruption of the brain-hepatopulmonary axis with impact on the liver, lung, and lungs and blood-brain barrier, where it may have mild neurocognitive consequences and behavioural consequences.
- Although increased plasma AGP, A2M and CRP may have beneficial effects in relation to the anti-microbial response, in relation to the systemic anti-microbial response, its inflammatory basis has one or more causes that must be one or more causes that need to be investigated, especially when they coincide in the patient with motility with alterations in GI motility in the patient. In fact, this hepatic response suggests persistence of viral antigens in the GI tract, as well as a bacterial dysbiosis with alterations in the perivascular bacterial dysbiosis with alterations in the permeability of the intestinal barrier and activation of neuro-immune based of neuro-immuno-inflammatory based mechanisms such as dyspepsia and irritable colon.
- In this respect, it should be recalled that the persistence of viral antigens in the GI tract leads to alterations of the GI tract leads to alterations of the intestinal physiology, which in turn generate alterations of the regional microbiota (bacterial dysbiosis) and intestinal permeability, with neuroinflammatory consequences with neuroinflammatory and neurocognitive consequences in the patient's brain via the hepato-pulmonary pathophysiological response.
- On the other hand, pathophysiological communication through the hepato-pulmonary axis is bidirectional and involves the parasympathetic nervous system, represented by the vagus. As a mixed interoceptive nerve - with afferent (80%) and efferent (20%) fibres - it constantly monitors gut health, sensing metabolites from the microbiota and regional immune system, whose information it transmits centrally to activate an anti-inflammatory cholinergic response that dampens GI inflammation and modulates the composition of the microbiota, helping to regularise altered intestinal permeability. However, when abnormal brain activation via the vagal route coincides with an alteration of the blood-brain barrier, excessive activation of microglia occurs, which has neuroinflammatory effects, in turn altering neuronal signalling pathways with possible neuropsychiatric and neurocognitive consequences that must be prevented and treated.

The following tables show the changes in the biological markers of damage to the encephalo-hepato-pulmonary axis (Figure 2).

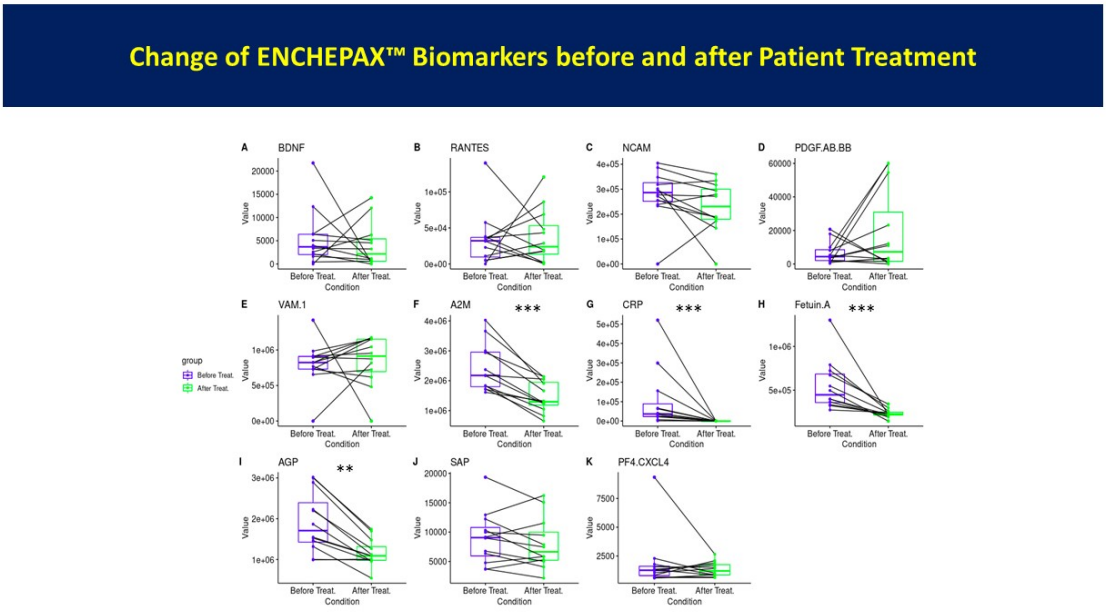

Figure 2.

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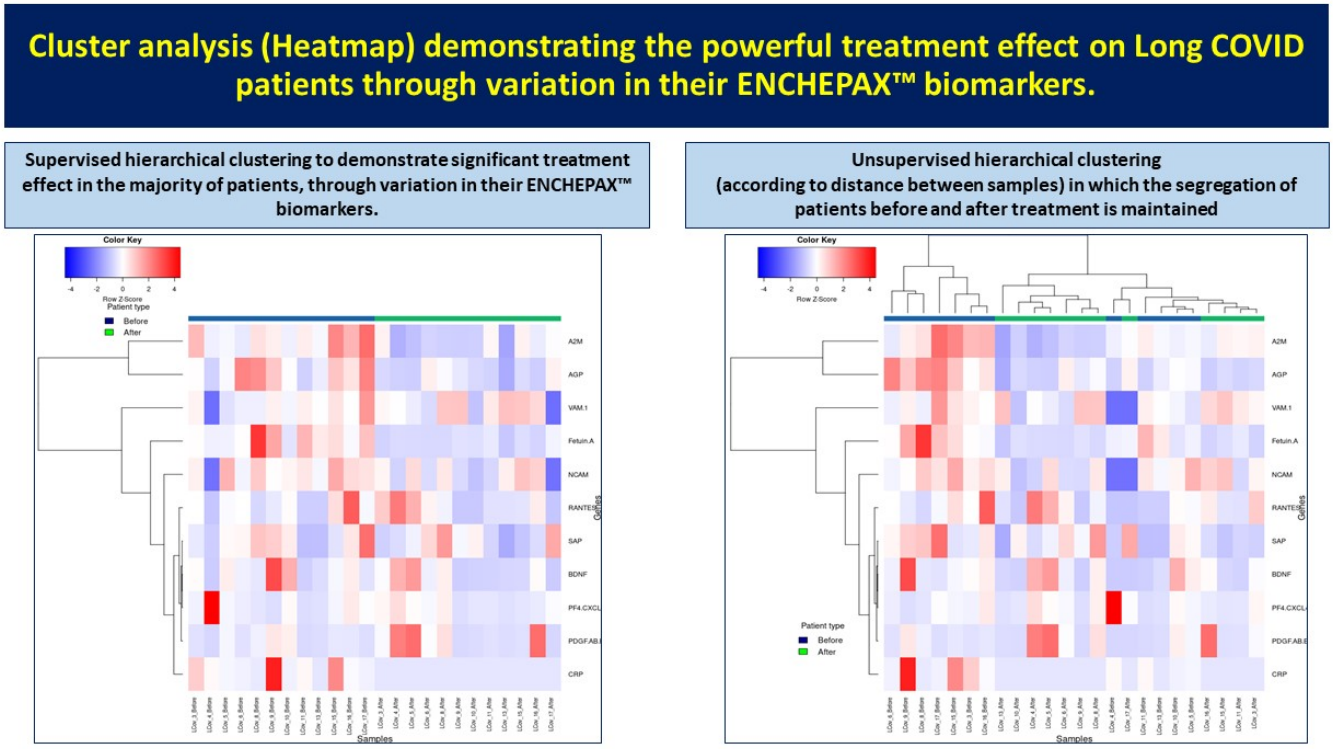

Figure 3.

Cluster analysis (Heatmap) to demonstrate the remarkable effect of VITAMIC BIOSEN® in LONG COVID patients, through variation in their ENCHEPAX™ biomarkers. Unsupervised hierarchical clustering according to distance between samples) where segregation of patients before and after treatment is maintained (Figure 3). Increased blood level of PF4/CXCL4 and PDGF-AB/BB discriminate most responsive patients to VITAMIC BIOSEN® (Figure 4).

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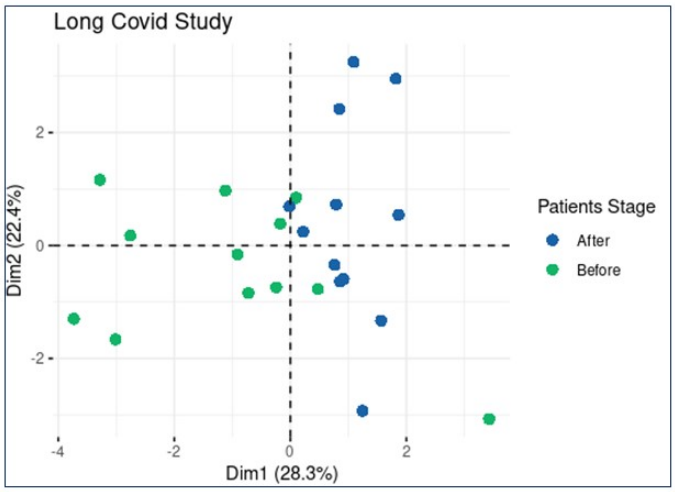
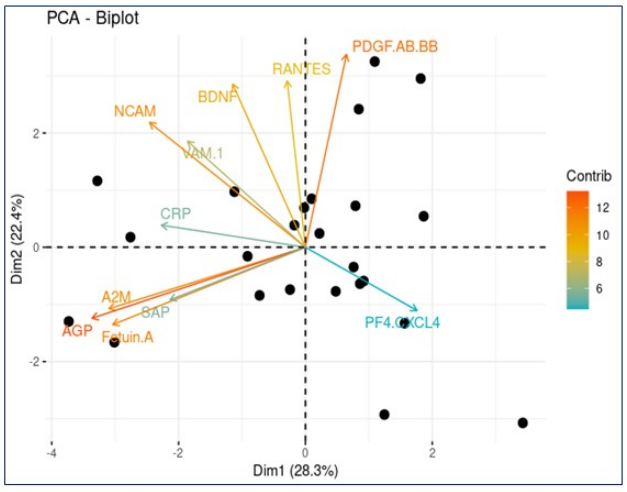
Principal Component Analysis to demonstrate the significant effect of treatment on Long COVID patients, through variation in plasma levels of their ENCHEPAX™ biomarkers.



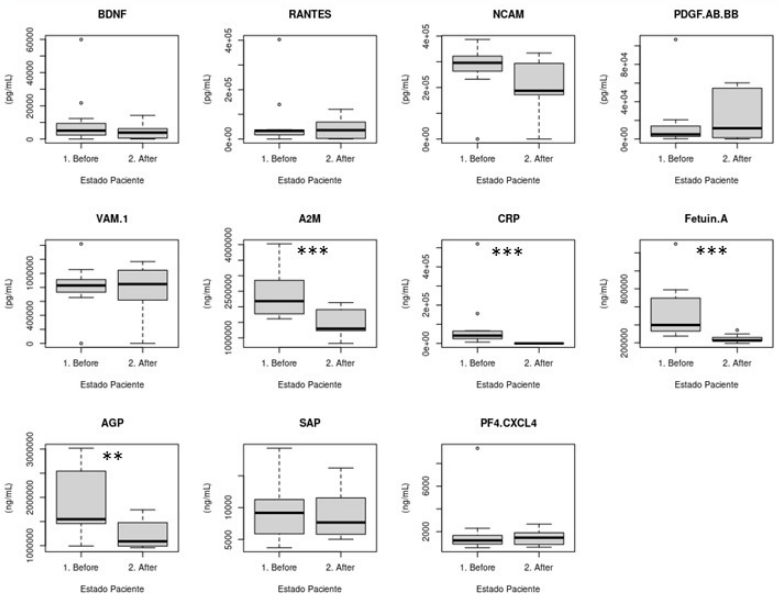


Taken together, the plasma concentration changes of the biomarkers are large enough to discriminate patients before and after treatment.

- **Increase in PF4/CXCL4 and PDGF-AB/BB**
- **Increased AGP, Fetuin A and A2M most discriminate patients before treatment.**

Figure 4.

Average ENCHEPAX™ Biomarker level before and after Patient Treatment



Striking impact of treatment on some of the ENCHEPAX™ biomarkers (A2M, CRP, Fetuin-A, AGP (Orosomucoid) in the majority of LONG COVID patients.

Figure 5.

High AGP, Fetuin A and A2M blood level discriminate patients before treatment who will respond to VITAMIC BIOSEN®. Principal Component Analysis to demonstrate the significant effect of VITAMIC BIOSEN® on LONG COVID patients, through variation in plasma levels of their ENCHEPAX™ biomarkers.

The plasma concentration pattern of immunoinflammatory molecular biomarkers discriminated patients before and after treatment with VITAMIC BIOSEN® (Figure 5). As shown by ENCHEPAX™ results, VITAMIC BIOSEN® remarkably affected the blood level of immunoinflammatory biomarkers from the liver (A2M, CRP, Fetuin-A, AGP / Orosomucoid) and the SAP (Serum amyloid A) majority of treated LONG COVID patients.

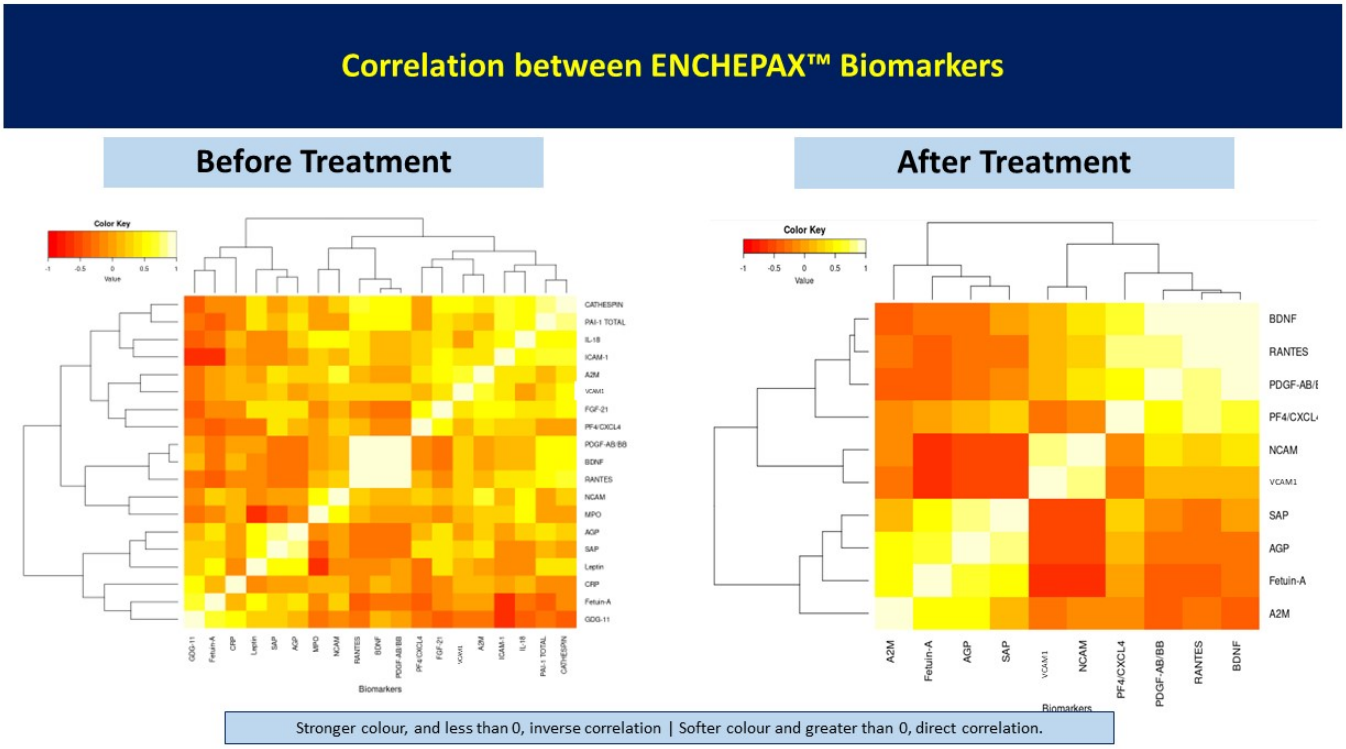


Figure 6.

The decline of SAP is relevant because in the pathophysiology of LONG COVID, amyloid protein has been found in microthrombi induced by the SPIKE protein of SARS CoV-2 as well as amyloid deposits have been described in the brain of SARS CoV-2 infected patients that would be related to the cognitive impairment symptoms associated with LONG COVID.

Dyspnea, sleep disorders, headache and depression significantly improved after VITAMIC BIOSEN®.

Pain, mobility problems and concentration problems were slightly improved (Figure 6 & Figure 7).

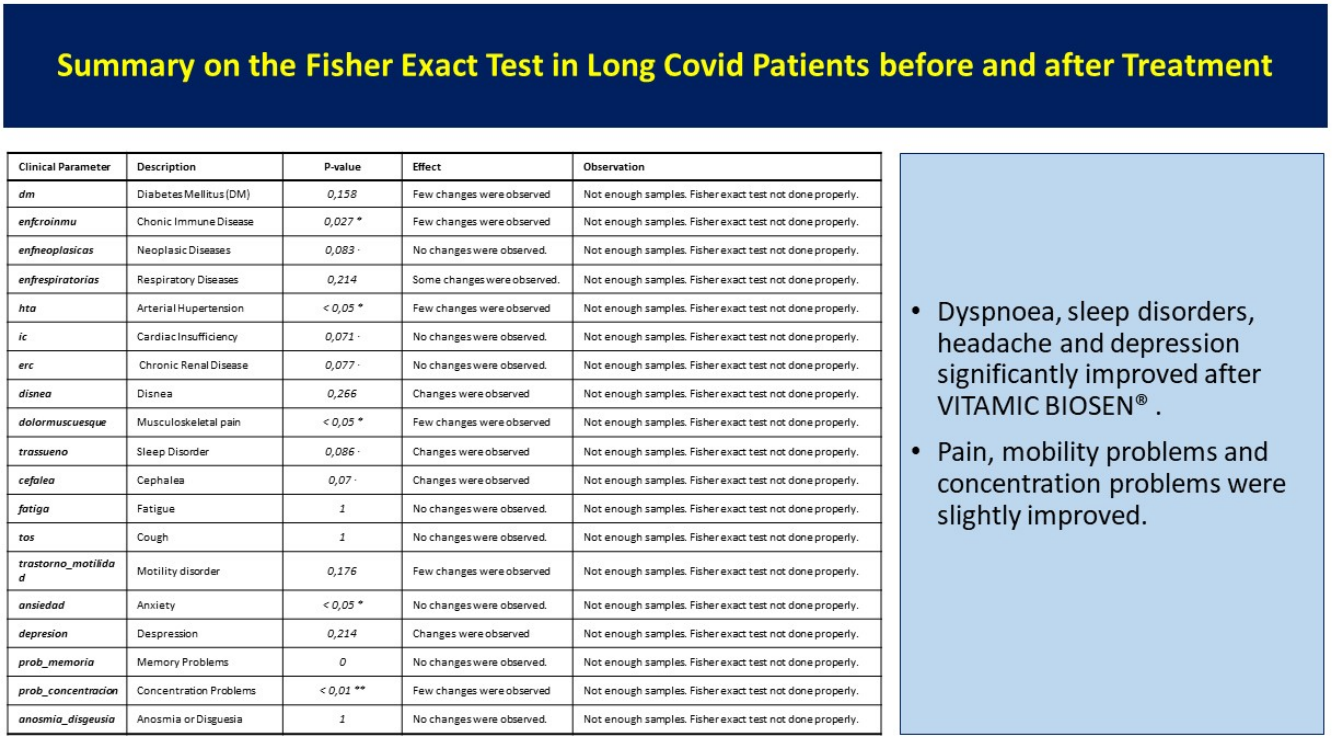


Figure 7.

Study has helped to biologically phenotype patients with LONG-COVID-like symptoms after vaccine immunization, studying the encephalo-hepato-pulmonary axis.

The study has shown beneficial effects in terms of clinical and biomarker reduction in LONG COVID patients given VITAMIC BIOSEN®

The study has helped to biologically phenotype patients with LONG-COVID-like symptoms after vaccine immunization.

Conclusion

This study had the following objectives:

1. To study the change in EuroQol5D Quality of Life Questionnaire. after one, two, three and six weeks.

2. To study the change in Post-Covid Functional Score (PCFS) after one, two, three and six week

3. To study the change in Post-Covid Symptomatology on a 10-point likert scale after one, two, three and six weeks.

- Dyspnea: we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

- Sleep disorders: we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

- Mobility intestinal problem. we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

- Depression we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

- Headache: we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

- Mental confusion: we have seen a significant difference in the number of times the symptom was reported (All visits with a downward trend).

VITAMIC BIOSEN® markedly affected the clinical symptoms of LONG COVID patients and modified their molecular pattern of immunoinflammatory biomarkers. The effect of VITAMIC BIOSEN® on immunoinflammatory molecular biomarkers could explain changes in the symptoms of LONG COVID patients. Determination of immunoinflammatory molecular biomarkers from the brain-hepatopulmonary axis not only serves to identify the LONG COVID patient's molecular endotype, but also LONG COVID

patients who need and will successfully respond to VITAMIC BIOSEN® effects. We observe a tendency of decrease in these symptoms especially when comparing the last visit with the rest. A percentage of patients showed improvement in asthenia and fatigue associated with the LONG COVID syndrome. There were 2 cases of special relevance, a patient who was on sick leave and was able to return to work, and a professional athlete who had not been able to compete or train for 6 months and 6 weeks after starting the treatment was able to compete in a high European competition and came second in a high impact race, a 31 km race with an elevation gain of 2200 metres.

https://www.marca.com/deportes-aventura/2022/03/16/6231d04546163f494d8b457e.html

https://www.marca.com/deportes-aventura/2022/08/13/62f7e3dbca4741112b8b456e.html

Ethics Report

This study (ACpn) was submitted to the ethical committee of the Clinico de San carlos Hospital Madrid.

Ethics Report

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