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STUDY OF VITAMIN D AND FERRITIN LEVELS IN SARS-COV-2 POSITIVE PATIENTS BY PCR AND THEIR RELATIONSHIP WITH THE ANTIBODY INDEX IgG.

Angel San Miguel Hernández*, María San Miguel Rodríguez, Julia San Miguel Rodríguez and Angel San Miguel Rodríguez

Clinical Analysis Service. Rio Hortega Valladolid University Hospital. International University of La Rioja (UNIR).

***Corresponding Author: Angel San Miguel Hernández**
Clinical Analysis Service. Rio Hortega Valladolid University Hospital. International University of La Rioja (UNIR).

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ABSTRACT

Currently, it is stated that patients with Covid-19 have low levels of vitamin D and high levels of ferritin in some of the phases of their disease. Vitamin D levels have been studied in 159 patients who initially tested positive for SARS-CoV-2 PCR and whose level of IgG antibodies to SARS-CoV-2 was measured in order to calculate their e index. try to see the relationship with vitamin D levels. The mean of the index values found against the IgG antibodies against SARS-CoV-2 of the 159 patients studied presented a value of 5.14 ±2.22. We have also observed that the mean of the vitamin D values found in these same patients presented a value of 19.7±8.3 ug/L. And the mean of the ferritin values found in the 91 patients presented a value of 956.6 ±405.2 ng/ml, with normal values in our laboratory of 10-120 ng/ml. In our study, we did not find statistically significant differences when comparing vitamin D levels with the SARS-CoV-2 antibody index by linear regression in the 159 patients studied ($p > 0.05$). Although we have observed in our case, that there is an important group of patients who have the highest levels of Vitamin D, with the lowest levels of antibodies against SARS-CoV-2, but without finding statistically significant differences. Nor have we found statistically significant differences when comparing ferritin levels with the SARS-CoV-2 antibody index, in the 91 patients studied ($p > 0.05$). Not even when we compared vitamin D levels with ferritin levels, we could not find significant differences in their correlation either. This could be due to the fact that our patients studied were not stratified according to clinical severity and were patients who were in hospitalization and in the ICU and in different phases of the disease. Covid-19 patients with lower vitamin D levels also have elevated serum levels of inflammatory markers such as ferritin and other markers. So if the treatment with vitamin D plays a role in the prevention of the disease or in the improvement of the prognosis of patients with Covid-19; this will have to be elucidated in large randomized controlled trials, as these studies are necessary to precisely define the role of supplementation with this vitamin in future waves of SARS-CoV-2 infection.

KEYWORDS: Coronavirus, SARS-CoV-2, vitamin D, ferritin.

INTRODUCTION

Covid-19 is a disease caused by the new coronavirus SARS-CoV-2, which was first identified in December 2019 during an outbreak of respiratory disease cases in China and that on March 11, 2020, the WHO declared the global pandemic by Covid-19.[1] The incubation period is 1 to 14 days, with most cases manifesting after 3 to 5 days.[2] The most common symptoms of Covid-19 are fever, tiredness, dry cough, and shortness of breath. A severe acute respiratory distress syndrome (ARDS) may develop.[3,4] Since the mortality rate is 6.3% worldwide4 and increases with age and the presence of comorbidities.

The causative agent of Covid-19 is a beta coronavirus and belongs to a family of viruses that can cause respiratory symptoms ranging from the common cold to severe pneumonia. These viruses are common in animals around the world and can affect humans, as has happened with SARS-CoV-2.[1]

SARS-CoV-2 is a virus belonging to the genus β-coronavirus, whose genetic material is a chain of ribonucleic acid (RNA). The genetic material of the virus of approximately 30 kb, is protected from the environment by four main structural proteins, which are: Protein E (envelope), is a transmembrane component of the virus, which is involved in the exchange of ions between the interior of the viral particle and the environment. It is also involved in the process of

entering the virus into the host cell through interactions with cell membrane proteins. Protein M (membrane), which is a glycoprotein that serves as the membrane of the viral particle and supports protein E. Protein M, is the most abundant in viral particles, and consists of three transmembrane domains. This protein is crucial in the assembly of viral particles, as it provides the scaffolding that gives the virus its shape and structure. Protein S (spike), which is a transmembrane glycoprotein that protrudes from the viral particle, having an ectodomain greater than 10 nm. This protein is made up of up to 300 monomers, whose S1 and S2 subunits are those that lead to the anchoring of the virus in the receptors of host cells. The protein N (nucleocapside), which is attached to the genetic material of the virus, forming the nucloecapside, which protects the RNA from the environment, and is essential for the release of RNA in the cytoplasm of the infected cell.

Along with genetic tests for the detection of SARS-CoV-2, there are serological tests that evaluate the appearance of antibodies generated by lymphocytic cells by virtue of exposure to antigens generated by viral subunits. In the case of SARS-CoV-2, the antibodies are generated for the antigens of the proteins N and S. These tests mainly measure immunoglobulins M and G, which appear in different stages of the disease and after the patient's recovery, at through immunological assays, such as ELISA. Serological tests are much less accurate than genetic tests, especially since immunoglobulins begin to appear 7 days after infection; although this period may vary depending on the severity of the infection. In contrast, RT-PCR and RT-LAMP methods can measure the pathogen from the first days of infection and in varied samples, such as mucosa, sputum or feces (Figure 1).[1-4]

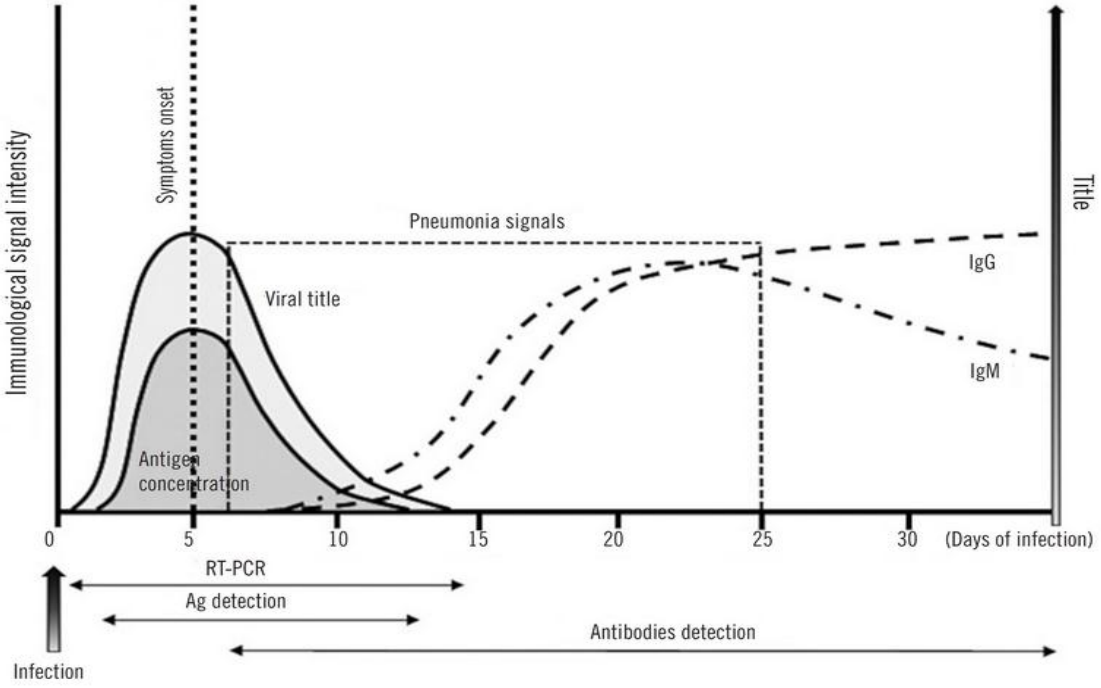

Figure 1. Diagram of laboratory tests and their evolution in the detection of SARS-CoV-2.

The immune system reacts to SARS-CoV-2 infection by producing specific antibodies. These antibodies appear in the serum or plasma of infected individuals after detection of viral ribonucleic acid (RNA) in smears[5] and from a few days to 2 weeks after the onset of symptoms.[6]

In addition, there are other laboratory parameters such as vitamin D, which is a fat-soluble steroid prohormone that is generated mainly in the skin by photochemical conversion of 7-dehydrocholesterol; It has two relevant biological forms, vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Both vitamin D3 and D2 can be absorbed from food, with vitamin D2 from an artificial source, although only 10% to 20% of vitamin D is acquired through intake[7] Vitamins D3 and D2 can be found in vitamin complexes. Vitamin D is converted to the active hormone 1,25-dihydroxyvitamin D (calcitriol) by two hydroxylation reactions. The first hydroxylation occurs in the liver and converts vitamin D to 25-hydroxyvitamin D. The second hydroxylation occurs in the kidneys and many other cells in the body and converts 25-hydroxyvitamin D to biologically active 1,25-dihydroxyvitamin D. Most cells express vitamin D receptors and about 3% of the human genome is directly or indirectly regulated by the endocrine system for vitamin D. 25-hydroxyvitamin D is the main storage form of vitamin D and is present in the blood in concentrations up to 1,000 times higher than the active form 1,25-dihydroxyvitamin D. 25-hydroxyvitamin D has a half-life of 2 to 3 weeks relative to the 4 hours of 1,25-dihydroxyvitamin D For this reason, 25-

hydroxyvitamin D is the analyte of choice for determining the status of vitamin D.[8,9]

Ferritin is also another useful laboratory marker, which is a large spherical protein composed of 24 subunits linked by non-covalent bonds, whose molecular weight is approximately 450,000.[10] The subunits form a shell surrounding a central core that contains varying amounts of ferric hydroxyphosphate.[11] One molecule of ferritin can bind between 4,000 and 5,000 iron atoms, making ferritin the main iron storage protein in the body.[12] It is found primarily in the cytoplasm of cells of the reticuloendothelial system, and it was previously believed that ferritin did not appear in plasma or extracellular fluid under normal conditions. However, the development by Addison et al. In 1972, a sensitive immunoradiometric technique resulted in the discovery that ferritin is present in all normal human sera.4 Through this and other studies, it was determined that ferritin concentration was directly proportional to total iron stores. of the body, with which ferritin levels became a common diagnostic tool in the assessment of iron levels.[13-15] In most normal adults, serum ferritin levels range from 10 to 300 ng/mL (µg/L), but concentrations vary according to age and sex.[10,13-15]

Traditionally, the calculation of stainable iron in bone marrow biopsies was the accepted method for assessing the body's iron stores. However, this method is traumatic for the patient and is only semi-quantitative. Other methods, such as serum iron determination, total iron-binding capacity (TIBC), and percent transferrin saturation, are subject to diurnal variations and are often imprecise. The latter methods also do not discriminate between depleted iron stores and conditions associated with defective iron release, such as anemia of chronic diseases.[16]

Today it is affirmed that patients with Covil-19 present in some of the phases low levels of vitamin D and high values of ferritin, in different stages of the disease.

OBJECTIVES

Vitamin D levels have been studied in 159 patients and ferritin levels in 91 patients who initially tested positive for SARS-CoV-2, confirmed by reverse transcriptase polymerase chain reaction [RT-PCR] and whose level of IgG antibodies to SARS-CoV-2 in order to calculate its index and try to see the relationship with vitamin D levels.

In addition, in the 159 patients studied, 91 were measured serum ferritin levels to try to see its correlation with the IgG antibody index and with vitamin D levels.

MATERIAL AND METHODS

We have studied 159 patients in whom the PCR was initially positive against SARS-CoV-2 and in whom the level of IgG antibodies against SARS-CoV-2 was measured, to later measure the levels of vitamin D in serum and try to see its correlation with the IgG antibody index.

The Abbott Diagnostics SARS-CoV-2 IgG Assay was used in an Architect, which is an automated two-step immunoassay for the qualitative detection of IgG antibodies to SARSCoV-2 virus in human serum and plasma using immunoassay technology. chemiluminescent microparticles (CMIA). The sample, SARS-CoV-2 antigen-coated paramagnetic microparticles, and assay diluent are combined and incubated. The IgG antibodies against SARS-CoV-2, present in the sample, bind to the microparticles coated with SARS-CoV-2 antigen. The mixture is washed. The acridinium-labeled anti-human IgG conjugate is added to create the reaction mixture and incubated. After a wash cycle, the pre-activator and activator solutions are added. The resulting chemiluminescent reaction is measured in relative light units (RLU). There is a directly proportional relationship between the amount of IgG antibodies against the SARS-CoV-2 virus in the sample and the URLs detected by the optical system and this relationship is reflected in the calculated Index (S/C), a sample/point index cutting. The presence or absence of IgG antibodies to the SARS-CoV-2 virus in the sample is determined by comparing the chemiluminescent URLs present in the reaction with the URLs of the calibrator.

The Architect iSystem calculation calculates the mean chemiluminescence signal of the calibrator from 3 replicates of the calibrator and stores the result. Results are expressed as the division of the sample result by the stored calibrator result. The units provided for the SARS-CoV-2 IgG assay results are Index (S/C), a sample/cut-off index. Interpretation of the results The cut-off point corresponds to an Index (S/C) of 1.4. According to the Index, a negative result for a patient is considered if it is <1.4 and positive if it is ≥ 1.4.

In addition, the Architect 25-OH Vitamin D assay from Abbott Diagnostics was used for chemiluminescent microparticle immunoassay (CMIA).

The mean values of the controls found in the Architect of vitamin D were: low control (n =15): intra-assay, 21.3±0.51 and inter-assay 21.7 ± 0.63, medium control (n =15): within assay 39.9±1.01, interassay 40.7 ±1.32 and high value (n = 15): within assay 77.4± 99, and interassay 80,2±2.47 ng/ml or ug/L. And the mean values of the HPLC controls were: low control (n =15): intra-assay, 18.5 ±0.78 and inter-assay 23.5±0.99, medium control (n =15): intra-assay 40.6 ±1.48, inter-assay 43.9 ±2.15 and high value (n = 15): intra-assay 79.8±2.89, and inter-assay 84.7±4.18 ng/ml or ug/ L.

The determination of ferritin has been carried out with the Access Ferritin assay, Beckman Coulter. The Access Ferritin assay is based on the two-site immunoradiometric assay (IRMA) described by Addison et al, but uses an enzyme-labeled antibody instead of the

radiolabeled tracer.[13] Ferritin measurement is very suitable for this assay method as its enormous size facilitates the simultaneous binding of the two or more required antibodies.

RESULTS

159 patients with ages between 19 and 79 years have been studied.

The mean of the mean IgG antibody index values in the 159 patients studied presented a value of 5.14±2.22.

Likewise, we have found that the mean vitamin D values in these same patients presented a value of 19.7 ±8.3 ug / L, with normal values in our laboratory being 10-50 ug/L.

Figure 2 shows the linear relationship of vitamin D values with the index of IgG antibodies against SARS-CoV-2 in 159 patients, in which a regression line was obtained from the study of the results of y = 19.6 - 0.15 x; and presented a correlation coefficient r2 of 0.008 (p> 0.05).

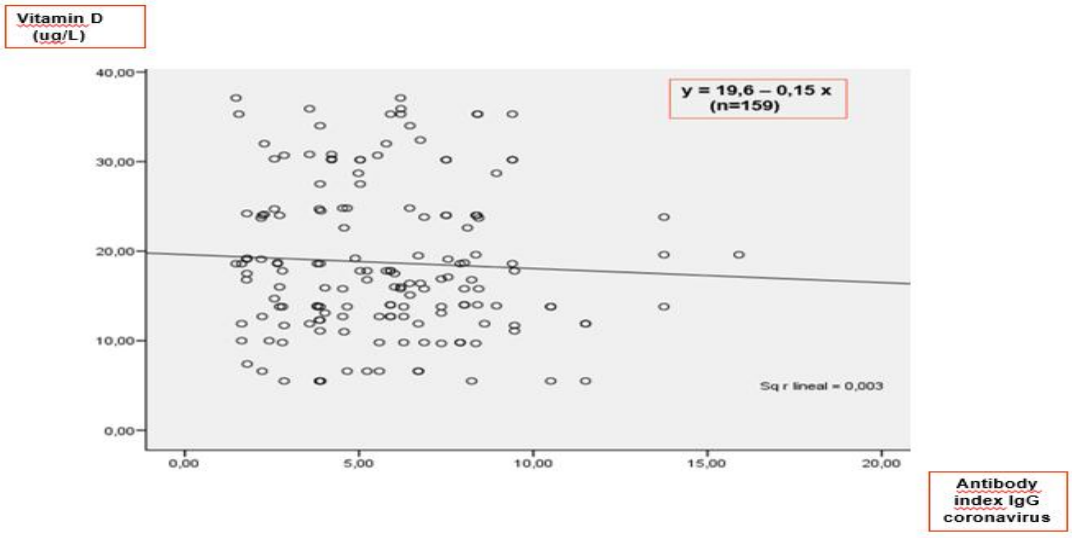

Figure 2: Linear relationship of vitamin D values with respect to the IgG antibody index against SARS-CoV-2 in the 159 patients studied.

They have also been studied from the 159 patients in whom it determined the vitamin D, to 91 in which the levels of ferritin in serum were determined.

The mean ferritin values found in the 91 patients presented a value of 956.6±405.2 ng / ml, with normal values in our laboratory of 10-120 ng / ml.

Figure 3 shows the ferritin values with the index of IgG antibodies against SARS-CoV-2, in which a regression line was obtained for the study of the results of y = 880 + 30.4 x; and presented a correlation coefficient r2 of 0.017 (p > 0.05).

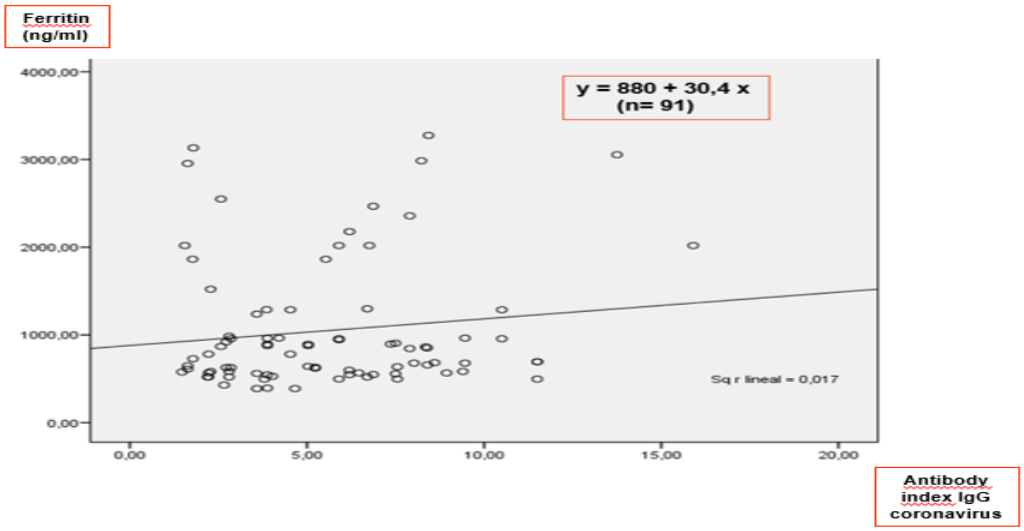

Figure 3: Linear relationship of ferritin values with the index of IgG antibodies against SARS-CoV-2, in the 91 patients studied.

An attempt was also made to correlate vitamin D levels with ferritin levels in 91 patients. Obtaining a regression line y = - 1147 - 5.38 x (n = 91), not obtaining statistical significance (p> 0.05) (Figure 4).

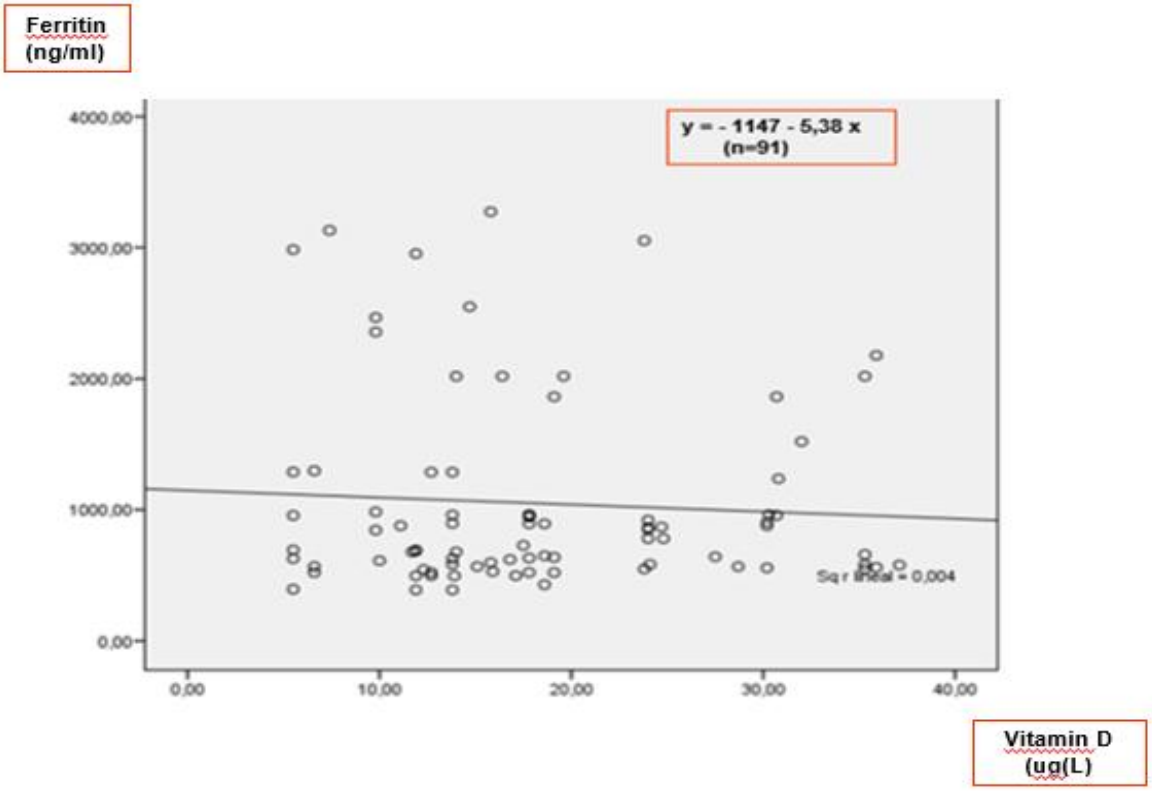

Figure 4: Linear relationship of vitamin D values and ferritin levels in the 91 patients studied.

Figure 5 shows schematically the mean values of the three markers measured in the study.

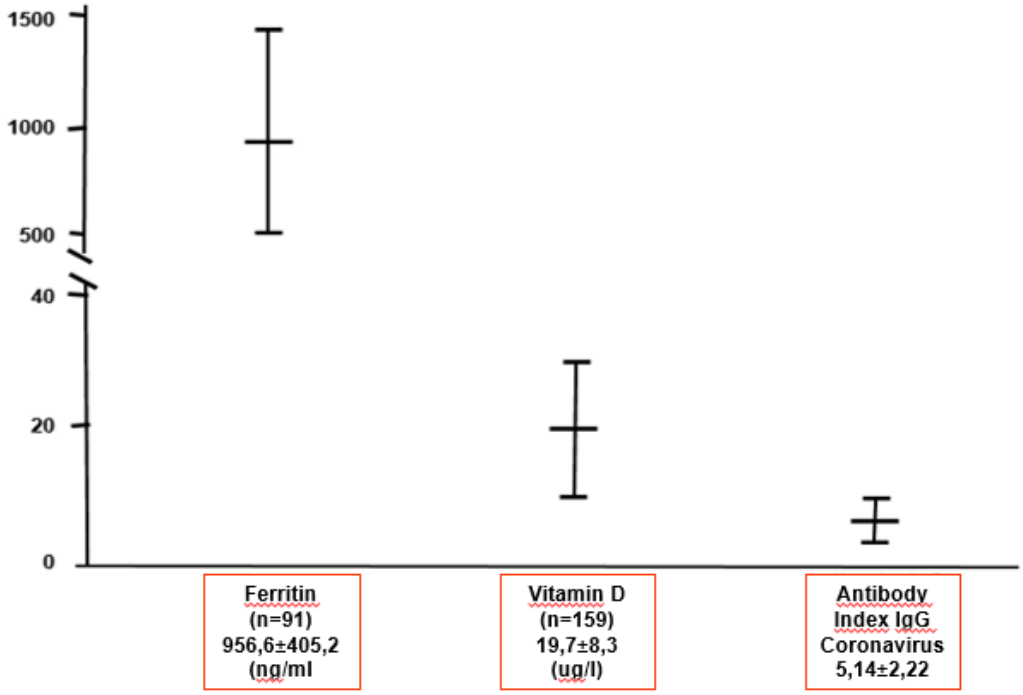

Figure 5: Mean levels of the different markers studied.

DISCUSSION

Index of Antibodies against SARS-CoV-2

We know that specific IgG antibodies against SARS-CoV-2 are detected in patients with Covid-19 during the symptomatic phase of the disease, after the RNA is no longer detectable.[5,6] The sensitivity of combining RNA results with antibody results is greater than 99%.[5] The persistence of IgG antibodies makes it possible to identify people who have been infected in the past, have recovered from the disease, and are immune. IgG detection and other serological tests will play an important role in the investigation and surveillance of this pandemic.[10]

Vitamin D

Epidemiological studies have shown a high global prevalence of vitamin D insufficiency and deficiency.[18] Risk factors for vitamin D deficiency include low sun exposure, malnutrition, some malabsorption syndromes, and liver or kidney disease. Measurements of vitamin D status provide opportunities to intervene in a preventive and therapeutic manner[19-21] Vitamin D deficiency is a cause of secondary hyperparathyroidism and of pathologies that cause alterations in bone metabolism, such as rickets, osteoporosis and osteomalacia.[8,22,23]

Although we lack sufficient evidence to justify supplementing with vitamin D in the prevention and / or treatment of Covid-19 infection, it is increasingly possible that this hypothesis is true. Since there are two basic mechanisms to consider. The anti-infective and immunomodulatory action that it exerts by improving intercellular barriers by stimulating innate immunity, as well as by modulating adaptive immunity. Also, vitamin D reduces the production of inflammatory cytokines such as IL-2 and interferon gamma (INF-γ). And multiple pleiotropic effects have been demonstrated on the actions of vitamin D at the anti-inflammatory and immunomodulatory level. Which explain the positive results in studies with influenza, coronavirus and other respiratory infections. An inverse relationship between serum vitamin D levels and the prevalence of respiratory infectious disease has been described. Another approach responds to considering the inhibition of the renin-angiotensin-aldosterone system, which is exacerbated in Covid-19 infection, because the virus binds to the ECA2 enzyme, leaving more angiotensin II available to cause damage. Vitamin D inhibits RAAS mediators, present in all cells of the body, and by inhibiting ACE activity and increasing ACE2, it decreases angiotensin II levels.[24]

In our case, we did not find statistically significant differences when comparing the linear relationship between vitamin D levels with the SARS-CoV-2 antibody index in the 159 patients studied ($p > 0.05$). Although we have observed that there is an important group of patients who have the highest levels of Vitamin D, with the lowest levels of antibodies against SARS-CoV-2, as can be seen in Figure 2 y 5.

Patients who have been hospitalized with Covid-19 and who have sufficient vitamin D, with a blood level of at least 30 ng/ml, had a significantly lower risk of adverse clinical outcomes including loss of consciousness, hypoxia and death. In addition, they had lower blood levels of an inflammatory marker C-reactive protein and higher levels of lymphocytes, immune cells that help fight infection. There is direct evidence that vitamin D sufficiency can reduce complications, including cytokine storm, meaning the release of too much protein into the blood too quickly, and ultimately death from Covid-19 of patients.[25, 26]

In patients over 40 years of age, it was observed that those patients who had enough vitamin D were 51.5% less likely to die from infection compared to patients who were deficient or deficient in vitamin D with a level of 25-hydroxyvitamin D blood less than 30 ng / mL. It was determined that a sufficient amount of vitamin D can reduce the risk of contracting the coronavirus by 54%, and it is believed that the fact that vitamin D is sufficient helps to combat the consequences of being infected not only with the coronavirus but also with other viruses that cause diseases of the upper respiratory tract, including influenza. Thus, a cost-effective strategy is provided to improve the ability to fight the virus and reduce the adverse outcomes of Covid-19, including the need for respiratory support, an overactive immune response that leads to a cytokine storm and death.[27-30]

Because vitamin D deficiency and insufficiency is so widespread in children and adults around the world, especially in the winter months, it is prudent for everyone to take a vitamin D supplement to reduce the risk of infection and complications from Covid- 19.[31]

According to other studies with vitamin D[26], in 323 patients with Covid-19, there is no evidence to recommend its prophylactic or therapeutic use. Furthermore, there is also no evidence to support the association between vitamin D levels and predisposition to Covid-19 or its severity. And due to the low sun exposure derived from confinement, in England vitamin D supplements are being recommended to prevent its deficiency, without this recommendation being related to respiratory infections.

In an observational study[33], he compared the levels of 25 (OH) D upon admission of 186, consecutive patients hospitalized with Covid-19 and confirmed by reverse transcriptase polymerase chain reaction [RT-PCR]) with the of a matched control group, by season of year, age and sex; of 2,717 patients (999 men and 1,718 women). The patients with Covid-19 had a lower median of vitamin D than the controls: 18.6 ng/mL, IQR, interquartile range 12.6-25.3, vs 21.5 ng/mL, IQR 13.9-30,8; $P = 0.0016$; and a higher percentage of vitamin D deficiency (25 (OH) $D < 20$ ng/ml): 58.6% vs 45.2%, $p = 0.0005$. A great sexual dimorphism was found, since this difference in vitamin D levels was only observed in men,

but not in women: 17.6 ng/ml, IQR 12.7-24.0 vs 20.3 ng/mL, IQR 13.7-28.3, P = 0.0234. In addition, in men the percentage of vitamin D deficiency was much higher in the cases with Covid-19 than in the controls: 67.0% vs 49.2%, p = 0.0006, this effect being more marked in patients with disease more advanced (based on radiological status findings). And vitamin D deficiency is claimed to be a possible risk factor for severe SARS-CoV-2 infection in men and advocate for the use of supplements. And in a study of control cases[34], in which 580 positive Covid-19 cases, hospitalized and outpatient, were compared with 723 negative Covid-19, it did not find this difference in the median of 25 (OH) D: 43.3 (IQR 32.1) nmol/l) vs 44.1 (IQR 31.2) nmol/l).

In other studies[35], 20 hospital patients with Covid-19 who had been determined for vitamin D were studied; although it is not specified when this determination was made. And vitamin D insufficiency was defined as <30 ng / ml. 13 patients required admission to the ICU. Of the patients who required ICU, 11 (84.6%) had vitamin D insufficiency compared to 4 (57.1%) of those who remained in the ward, without the difference being significant (p = 0.29).

Since vitamin D is a substance that helps the body absorb calcium and is necessary for the formation of bones, its deficiency can lead to osteoporosis and rickets. In addition, it plays an important role in the nervous, muscular and immune systems, key in the fight against Covid-19. And there are different chronic diseases that have been directly linked to vitamin D depletion, especially diabetes mellitus 1, rheumatoid arthritis, multiple sclerosis, Crohn's disease, psoriasis, prostate, breast, ovarian and cancer. colon, hypertension and metabolic bone disease. And that the main effects of vitamin D deficiency occur in the bone; in children, it gives rise to rickets with growth retardation and deformities in the long bones, and in adults, it is the cause of osteomalacia characterized by lower bone density and increased risk of fracture.

During confinement, in countries with few hours of light or the winter season, with less time of sun, these are situations that can decrease the levels of vitamin D. And this is due to the fact that the human body synthesizes cholecalciferol (vitamin D3) in the skin by the action of ultraviolet light. Thus, sunbathing every day for 5-15 minutes not only improves our mood and generates a feeling of well-being, but also ultraviolet radiation is absolutely necessary for our body to synthesize vitamin D. If we are prudent, we can expose ourselves to it. Sun without experiencing risk from a window or although babies, people with fair and sensitive skin or those who have shown some sign of sun damage should not be exposed directly. It is usually enough to expose the face and arms, although it is advisable to rotate the exposed areas, since chronic sun damage causes premature aging of the skin, leading to the appearance of spots and wrinkles, as well as different forms of skin cancer.

Thus, hospitalized Covid-19 patients who had sufficient vitamin D, with a 25-hydroxyvitamin D blood level of at least 30 ng / ml, had a significantly lower risk of adverse clinical outcomes including unconsciousness, hypoxia, and death. In addition, they had lower blood levels of an inflammatory marker (C-reactive protein) and higher levels of lymphocytes. This provides direct evidence that vitamin D sufficiency can reduce complications, including cytokine storm, which involves releasing too many proteins into the blood too quickly and ultimately death from Covid-19.

In this sense, the administration of calcifediol, which is the most active metabolite of the endocrine system of vitamin D to hospitalized Covid-19 patients, greatly reduces their need for ICU admission. They reveal that vitamin D contributes to reducing the acute respiratory distress syndrome produced by Covid-19 and that calcifediol may be able to reduce the severity of the disease.

Experts agree that the differential factor in the treatment that US President Donald Trump received was a cocktail of monoclonal antibodies in an experimental phase from the Regeneron company. But, in addition, he was also given other substances such as zinc, vitamin D, famotidine, melatonin, aspirin, remdesivir and dexamethasone.

People who live in a state of confinement or in countries with little sun are at greater risk of presenting a vitamin D deficiency and, in these cases, it is advisable to increase their intake through food or dietary supplements. Among the recommended foods with a contribution of Vitamin D we have, the fatty acids of the fish represent the richest source of cholecalciferol, being the salmon the main source; eggs, butter, liver and other organ meats as well, but you have to watch out for their consumption due to their high cholesterol content. Other products with vitamin D are tuna, sardines; oysters, prawns and prawns; mushrooms; and avocado.

More than 80% of Covid-19 patients are deficient in vitamin D, and this deficiency is more common in men. As vitamin D is a hormone produced by the kidneys that controls the concentration of calcium in the blood and affects the immune system and its deficiency has been linked to a large number of problems. Also, more and more studies point to the beneficial effect of this vitamin D on the immune system, especially with regard to protection against infections.

Therefore, vitamin D deficiency should be identified and treated, especially in those groups at high risk of Covid-19 disease, such as the elderly or patients with comorbidities. Treatment with vitamin D should be recommended for Covid-19 patients with low levels of this hormone in the blood, as it could have beneficial effects on both the musculoskeletal and immune systems. Covid-19 patients with lower vitamin D levels also have

elevated serum levels of inflammatory markers such as ferritin and D-dimer, a marker related to blood clotting problems. And no relationship was found between vitamin D concentrations or deficiency and the severity of the disease.

So whether treatment with vitamin D plays any role in preventing the disease or improving the prognosis of patients with Covid-19, it will have to be elucidated in large randomized controlled trials. These types of studies are necessary to precisely define the role of supplementation with this vitamin in future waves of SARS-CoV-2 infection.

Ferritin

Currently, serum ferritin levels are useful as a tool to monitor the effects of iron therapy, although the results must be interpreted with caution, since ferritin levels in these cases may not always reflect the true state of iron stores. iron. In both adults and children, chronic inflammation results in a disproportionate increase in ferritin levels relative to iron stores. Elevated ferritin levels are also observed in cases of acute and chronic liver disease, chronic kidney failure, and in some types of neoplastic pathology.[36-38]

It has been suggested that high ferritin levels may indicate a progression to a severe Covid-19 infection. High ferritin concentrations would be associated with increased production of special signaling molecules, which can cause complications and lead to death. Since in approximately half of the cases, patients with high ferritin levels may die, as observed in the Covid-19 infection, which is reminiscent of the situation with hyperferritinemic syndrome.

Elevated levels of ferritin indicate the presence of pathogens in the body, but it can also be caused by a genetic mutation. And in the latter case, they can lead to neurological disorders and vision problems.

Different studies have shown that ferritin can activate macrophages, which are a type of white blood cell in the immune system that plays an important role in innate immunity, and is the body's first line of defense. This is evidenced by hyperferritinemic in patients with septic shock, antiphospholipid syndrome, and other conditions characterized by macrophage activation. When these are activated, they begin to secrete cytokines, which are signaling molecules that mediate and regulate immunity. At low concentrations, they are safe for the body and help protect it against pathogens. At high levels, the cytokine storm develops, which can be lethal for half of the patients, especially the elderly. Therefore, hyperferritinemic has been associated with greater severity of the disease and adverse outcomes, as in the case of Covid-19.

Other Bookmarks

Another important marker of macrophage activation is CD163, which has a high probability of complications and therefore, possible methods to inhibit the synthesis of CD163 and other macrophage signaling molecules using antibodies are being studied.

Evidence suggests that some Covid-19-infected patients may respond with a cytochemical storm with characteristics similar to bacterial sepsis or hemophagocytic/lymphohistiocytosis syndrome. This progression could explain the clinical phenomenon in which patients are relatively stable for several days, but suddenly deteriorate when they enter the adaptive immunity stage.[39-41] The existence of predictors of poor prognosis could be useful to detect patients who require individualized attention and targeted and early therapy.

According to the data available to date, among hospitalized patients, approximately 10-20% of patients require ICU, 3-10% require intubation, and 2-5% of patients die.

Predictors of fatality from a recent retrospective, multicenter study of 150 confirmed Covid-19 patients in Wuhan (China) included elevated ferritin (mean 1297.6 ng / ml in non-survivors vs 614.0 ng / ml in survivors; p <0.001) and IL-6> 7 (p <0.0001), suggesting that the higher mortality could be driven by hyperinflammation. Other clinical and analytical parameters that show significant differences between patients admitted to ICU versus non-ICU are: Respiratory rate greater than 24 resp/min, Lymphocytes less than 1 x 10 **9**, Prothrombin time greater than 12 seconds, D-dimer (mg/dl) greater than 2.4, Albumin (g/L) less than 30, ALT and ALT (U/L) greater than 40, LDH (U/L) greater than 248, ferritin greater than 800, saturation on admission < 905.[42,43]

Previous studies have suggested that lymphopenia and cytochemical storm are typical alterations in infections caused by highly pathogenic coronaviruses such as SARS and MERS.[40,44-46]

Thus, a decrease in the lymphocyte count and an increase in inflammatory cytokines in peripheral blood has been reported in Covid-19 infection.[46,47,48]

Given the rapid expansion and high mortality, a better knowledge of the clinical characteristics is mandatory to be able to carry out a scan of inflammation markers and to be monitored during the course of the disease.[44,48,49]

Authors such as Metha et al.[50] suggest that in patients with severe Covid-19 infection, the state of hyperinflammation should be ruled out through laboratory markers, such as increased ferritin, decreased platelets, increased ESR and the HScore to identify the subgroup of patients who could benefit from immunosuppression and mortality could decrease. Therapeutic options such as corticosteroids, immunoglobulins or interleukin blockers (tocilizumab or

anakinra) and JAK inhibitors are possible in these hyperinflammatory situations.

Studies have shown that in the pathogenesis of SARS-COV-2, an inflammatory storm occurs that produces a considerable release of pro-inflammatory cytokines including (IL-6, TNF-α and IL-12).[51]

Like the changes produced in SARS and MERS, in Covid-19, there are high levels of cytokines in plasma including IL-6, IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A and TNF-α that have been detected in patients admitted to the ICU[42,52] and are related to the severity and prognosis of the disease. In the biopsy of autopsy samples from a patient who died of severe Covid-19 infection, histological analyzes show diffuse alveolar involvement with fibromyxoid exudate, with mononuclear lymphocytic infiltrate in both lungs.[53]

Both GM-CSF and IL-6 are the main cytokines that support the inflammatory storm that could lead to a dysfunction in the alveolar-capillary exchange, altering diffusion, producing pulmonary fibrosis and organ failure.[54] Studies suggest that IL-6 could play a key role in this cytochemical storm.[55,56]

Liu et al.[46], studied a total of 69 seriously ill patients infected by Covid-19 compared with patients without severe affectation, assessing the immunological parameters and cytokines. They detected a slight variation in IL-2, IL-4, IL-10, TNF-a, IFN-γ before and after treatment. Elevated CRP, ferritin, IL-6 and LDH were associated with the need for a more intensive and prolonged treatment which included corticosteroids, immunoglobulins, antibiotics, oxygen therapy and mechanical ventilation. This fact shows that these analytical parameters are related to the severity of the disease. Other studies showed that changes in IL-6 were related to disease, suggesting that IL-6 could be a useful tool in monitoring severe Covid-19 disease.

A structured approach to the clinical phenotype is imperative to distinguish in the phase where viral pathogenicity is dominant versus the host's inflammatory response. Hasan et al., propose a clinical staging system to establish a standardized nomenclature for the uniform evaluation and notification of this disease, to facilitate the therapeutic application and evaluate the response. They propose the use of a 3-stage classification system, recognizing that Covid-19 disease exhibits three degrees of increasing severity that correspond to different clinical findings, response to therapy, and clinical outcome.

-Stage I. Diagnosis at this stage includes PCR of respiratory specimen, serum testing for SARS-CoV-2 IgG and IgM, along with chest imaging, complete blood count, and liver function tests. Laboratory tests can reveal lymphopenia and neutrophilia without other significant abnormalities.

-Stadium II. During this stage, patients develop viral pneumonia, with cough, fever, and possibly hypoxia (defined as a PaO2/FiO2 of <300 mmHg). Chest X-ray or CT images reveal bilateral infiltrates or ground glass opacities. Blood test reveals increased lymphopenia, along with increased transaminases. Markers of systemic inflammation may be elevated, but not excessively. It is at this stage that most Covid-19 patients would need to be hospitalized for further observation and treatment.
-Stage IIa (without hypoxia) and Stage IIb (with hypoxia).
-Stadium III. Covid-19 infection causes a decrease in helper, suppressor, and regulatory T cell counts. Several studies have shown that inflammatory cytokines and biomarkers such as interleukins (IL-2, IL-6, IL-7), granulocyte colony stimulating factor, macrophage inflammatory protein 1-α, tumor necrosis factor α , C-reactive protein, ferritin, and D-dimer are significantly elevated in patients with more severe disease.

Troponin and N-terminal B-type natriuretic peptide (NT-proBNP) may also be elevated. Since a form similar to hemophagocytic lymphohistiocytosis, it can occur in patients in this advanced stage of the disease. At this stage, shock, vascular spasms, respiratory failure, and even cardiopulmonary collapse can be detected. Systemic organ involvement, including myocarditis, would manifest during this stage. Personalized therapy in stage III relies on the use of immunomodulatory agents to reduce systemic inflammation before the patient ends in multi-organ dysfunction.

Among the laboratory data that are significantly different between patients with severe Covid-19 and patients with non-severe forms of the disease and that are, in most cases, the main predictors of the severity of the disease. Covid-19, we have elevated D-dimer and lymphopenia, which are also associated with higher mortality; as well as LDH, thrombocytopenia, C-reactive protein (CRP), and ferritin.

In the technical information document of the Ministry of Health[57], the laboratory findings and their possible relationship with the greater severity of Covid-19 cases are described. It is commented that among the parameters measured at the time of admission, together with age and comorbidity, the SOFA index (Sequential Organ Failure Assessment) can also predict mortality; odds ratio [OR]: 5.65; 95% CI: 2.61–12.23; p <0.0001), and the D-dimer greater than 1 microg / mL (OR: 18.42; 95% CI: 2.64–128.55; p = 0.0033), which is a prognostic evaluation scale of sequential organ failure that is composed of the sum of the score obtained after the evaluation of six organs or systems, such as the central nervous system, respiratory, liver, kidney, cardiovascular and coagulation.

In the CDC Guidelines[58], it is indicated that age is an important risk factor for severe disease, complications and death and that the cases of death were also higher for

patients with comorbidities. Regarding the laboratory findings, it stands out that lymphopenia, neutrophilia, elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), elevated lactate dehydrogenase (LDH), elevated C-reactive protein, and elevated ferritin levels may be associated with increased severity of the disease and also that elevated D-dimer and lymphopenia have been associated with mortality.

In addition, the evidence from the BMJ Best Practice[59] establishes that factors associated with disease progression and a worse prognosis include advanced age, a history of smoking, maximum body temperature at admission, respiratory failure, and a significant decrease in albumin level serum and significantly elevated protein C reactive. It adds that thrombocytopenia has been associated with an increased risk of severe disease and mortality, and may be useful as a clinical indicator to monitor disease progression, and that other factors associated with a poor prognosis include a higher score on the SOFA index and a D-dimer level greater than 1 mg / L.

In Uptodate's evidence on Covid-19[60], he lists as laboratory findings that have been associated with worse clinical outcomes: lymphopenia; elevated liver enzymes; elevation of LDH; elevation of elevated inflammatory markers (C-reactive protein, ferritin); elevated D-dimer (greater than 1 mcg / mL); a high prothrombin time (PT); elevated troponin; elevated creatine phosphokinase (CPK); and acute kidney damage. A progressive decrease in lymphocyte count and an increase in D-dimer were observed in patients who did not survive compared to the maintenance of more stable levels in patients who did not die.

At the Iberoamerican Cochrane Center, the prognostic value of D-dimer is specifically evaluated.[61] Data were collected that provide series of patients with Covid-19 that describe what analytical data are associated with the severity of the disease and, after reviewing five studies, in which D-dimer values were offered for both patients with a mild and severe evolution of the disease, concludes that: In the main series of Covid-19 patients published, patients with a worse prognosis have shown much higher D-dimer levels than patients with less severe disease. In a series of patients, a D-dimer value at the time of admission greater than 1.0 mg/L was one of the main factors of poor prognosis. In these studies, a higher PT has also been observed among the most seriously ill patients. They also conclude that D-dimer is associated with the severity of Covid-19, the authors of a pooled analysis[62], affirm that the review carried out shows that D-dimer values are higher in patients with severe Covid-19 than in those with milder forms and, therefore, D-dimer measurement may be associated with the evolution towards a worse clinical picture.

In a systematic review[63] that included 32 studies and a total of 4,789 patients, it detected several factors that contribute to a worse outcome, including old age, male gender, the presence of underlying diseases and some abnormal laboratory findings, such as a High D-dimer value He further notes that some studies suggest that lymphopenia and thrombocytopenia in patients with Covid-19 are also associated with worse clinical outcomes.

One of the systematic reviews[64], on risk factors for severe Covid-19, included 20 observational studies with 4,062 patients. Regarding the laboratory findings, the review observes that in seriously ill patients the white blood cell count increased, weighted mean difference [WMD] = 1.76 [0.31, 3.22]), while the proportion of lymphocytes decreased (WMD = -0.42 [-0.52, -0.33]. Compared with non-serious patients, seriously ill patients had higher levels of ALT, AST and total bilirubin (BT), ALT: WMD = 16.97 [2.18, 31.76]); AST: WMD = 20.60 [6.81, 34.40]; BT: WMD = 3.93 [2.01, 5.86], but there were no differences in creatinine, albumin or neutrophil count). With regard to C-reactive protein and procalcitonin (PCT), their values were higher in patients with disease than in non-severe patients (C-reactive protein: standardized mean difference [SMD] = 2.11 [0.71, 3.51]; PCT: WMD = 0.16 [0.04, 0.28]). Furthermore, the study found that, compared to non-seriously ill patients, LDH, CPK, and D-dimer increased more significantly in severely ill patients (LDH: SMD = 2.0 [1.20, 2.80], CPK: WMD = 23.55 [17.08, 30.02], D-dimer: DMP = 0.67 [0.02, 1.32]).

Another systematic review[65], carried out with the objective of describing the prognostic factors of Covid-19, analyzed 30 observational studies that included 53,000 patients. Clinical and laboratory data from 26 studies, including 1,374 critically ill and 4,326 non-critically ill, were extracted for meta-analysis. In terms of laboratory results, there were obvious differences between severe and non-severe cases in platelet count (mean difference [MD] = -30.654 × 109 / L; 95% CI: -38.7 to -22.61 ; n = 8), in lymphocyte count (MD = -0.376 × 10**9** /L; 95% CI: - 0.467 to - 0.285; n = 11), LDH (DM = 150,702 U/L; 95% CI: 82,569 a 218,836; n = 5), in the value of D-dimer (MD = 0.715 mg/L; 95% CI: 0.562 to 0.868; n = 7) and of C-reactive protein (MD = 30,395 mg / L; 95 CI %: 20,006 to 40,784; n = 10). ORs were calculated for various indices, including lymphocytopenia (OR = 4.23, 95% CI: 3.03-6.03), thrombocytopenia (OR = 2.84, 95% CI: 2.00-4.04), elevated D-dimer (OR = 3.17; 95% CI: 1.86-5.41) and elevated C-reactive protein (OR = 4.23; 95% CI: 2.94-6.08).

A large series of cases[66], carried out in New York, evaluated the factors associated with hospitalization and serious illness in 4,103 patients with Covid-19, of which 1,999 (48.7%) were hospitalized and 650 of them experienced a severe form of the disease. The factors associated with the evolution to severe disease were oxygen saturation at admission <88% (OR 6.99; 95% CI:

4.5 -11.0), and values in the first D-dimer test > 2,500 ng / mL (OR 6.9; 95% CI: 3.2 -15.2), ferritin> 2,500 ng / mL (OR 6.9; 95% CI: 3.2-15.2), and C-reactive protein > 200 mg / L (OR 5.78; 95% CI: 2.6 -13.8).

The authors of a multicenter retrospective study (67), with data from a sample of 208 patients with Covid-19, of which 40 [19.2%] showed progressive deterioration of their clinical condition during the observation period, a scale prediction of risk of worsening of Covid-19 (CALL score). They compared the clinical characteristics of the group of patients who remained stable and the group with progressive deterioration. Age, presence of comorbidity, lymphocyte count, and D-dimer and LDH values were significantly different between the two groups, but the multivariate analysis showed that comorbidity (hazard ratio [HR] 3.9; CI 95% 1.9 -7.9), age > 60 years (HR 3.0; 95% CI 1. -6.0), lymphocyte count ≤ 1.0 × 109 / L (HR 3,7; 95% CI 1.8-7.8), LDH 250-500 U/L HR 2.5; 95% CI 1.2-5.2) and LDH> 500 U / L (HR 9.8; 95% CI 2.8 - 33.8) were independent high-risk factors for worsening Covid-19. And to facilitate the clinical use of this information, the prediction model called CALL, comorbidity, age, lymphocytes and LDH was established, with a score of 4 to 13 points.

The CALL scores were classified into 3 risk levels according to their probabilities of progression to higher severity: those with 4-6 points had less than 10% probability of worsening; with 7-9 points they had between 10% and 40% probability of worsening; and with 10-13 points the probability of worsening was high (> 50%). Using a 6-point cut-off value, the positive predictive values (95% CI) were 50.7% (38.9%-62.4%) and the negative predictive values (95% CI) were 98.5% (94.7-99.8%); the positive and negative likelihood ratios were 4.31 (3.20-5.80) and 0.06 (0.02-0.20), respectively.

In genetically predisposed patients, Covid-19 would activate the macrophage and produce macrophage activation syndrome. This would trigger a cytokine storm, especially IL 6. This storm would be the one that causes patients to go bad, especially the younger ones. Therefore, high-dose corticosteroids are used in those with this macrophage activation syndrome. Since the patient who has the highest ferritin, the state of it will be worse with a worse prognosis. Since high ferritin levels are indeed associated with an acute inflammatory response, which appears in many seriously ill patients. Therefore, it is an indicator that the patient is going to get very worse, since non-serious patients also have very high levels, and a cut-off point should be established to determine which levels are very high and are associated with a bad progression and which levels are high, but are not associated with it.

Some people they are asymptomatic to Covid-19, but with up to 20% of patients, the body of some individuals, who hours before were not serious, can reach the limit of collapse. When Covid-19 enters the body, the individual initiates the response against it by sending macrophages, large cells that absorb the coronavirus and degrade it, sending a signal to the rest of the body. Most of the time it works and our body defeats the pathogen, but when these macrophages fail to neutralize the advance of the virus, they begin to send signals to the rest of the organism; cytokines are those banners that warn that something is wrong, producing a cascade of reactions in the rest of the body, usually to reduce inflammation.

When there is a cascade release of these cytokines to all parts of the body. Every site they go to, more macrophages are produced and they secrete more cytokines, which ends with the exhausted immune system and the virus will continue to advance. And among the laboratory parameters to predict it, there are high levels of ferritin and lymphopenia, which would be the white blood cells that should fight the virus in the blood.

Therefore, all the ferritin in the blood of people who have not overcome the infection by Covid-19, comes from macrophages infected by the virus, specifically those capable of targeting a receptor, CD-163, the same one that intervenes in the inflammatory response to respiratory diseases such as asthma and chronic obstructive pulmonary disease or COPD.

The virus attacks causing pneumonia and the body reacts by unleashing the fatal release of cytokines. In its initial phase, it is capable of evading the action of the immune system, which should be led by lymphocytes, and this initial inhibition allows the virus to cause direct damage to lung tissue. This first damage activates another type of response of the immune system, mediated by the generation of various cytokines that leads to the final activation of the macrophage pathway; This response is what generates a large increase in the pulmonary inflammatory process that ultimately produces respiratory failure.

As the current treatment of Covid-19 is supportive, and respiratory failure due to acute respiratory distress syndrome (ARDS) is the leading cause of mortality and among blood tests to detect those high levels of ferritin or IL-6 that could be determining the proximity of the storm in these patients. The mortality could be due to a hyperinflammation driven by the virus.

Currently, the treatment would be to administer anticoagulants to avoid the thrombi that often appear, but it does not stop being prevention until the severity of the infection subsides. Thromboembolic disease plays a very important role in the pathophysiology of Covid-19, but there are also other factors that cause kidney, respiratory, and heart function to be altered, as there are some cases that can reduce viral load. Since it is true that severe cases of Covid-19 present with very serious coagulation disorders and thrombotic phenomena, exactly as in any situation of sepsis. Thus, all septic shock situations end

in this way, or in renal failure, but what is clear is that the fundamental problem is pneumonia, although it is also true that the autoimmune phenomenon has similar peculiarities, in the final stages; since it would not be pneumonia the only one that causes damage, but the immune system itself that fights against oneself.

It has been suggested that elevated levels of five serum markers can be used to identify Covid-19 patients who are at high risk of serious complications or death. Based on the studies that showed that certain biomarkers were associated with poor results, they determined the levels of these in samples obtained from 299 patients diagnosed with Covid-19, among which are.

- D-dimer. It is an important test in patients with suspected thrombotic disorders, since a four-fold increase in protein is a strong indicator of mortality in Covid-19 patients.
- C-reactive protein (CRP) is an acute phase protein, of hepatic origin, which increases after the secretion of interleukin-6 by macrophages and T cells.
- IL-6 (interleukin-6), which is an important mediator of fever and the acute phase response. There is some preliminary evidence that IL-6 can be used as an inflammatory marker for severe Covid-19 infection with poor prognosis, in the context of a broader coronavirus pandemic.
- Lactate dehydrogenase (LDH), which is an enzyme found in almost all living cells. It is widely expressed in body tissues, such as blood cells and heart muscle. Since it is released during tissue damage, LDH is a marker for common injuries and diseases such as heart failure.
- Ferritin, which is a universal intracellular protein that stores iron and releases it in a controlled manner. Plasma ferritin is an indirect marker of the total amount of iron stored in the body and is used as a diagnostic test for iron deficiency anemia.

The results have revealed that elevated levels of these markers are associated with inflammation and bleeding disorder, showing an increased risk, independent of ICU admission, of invasive ventilatory support and death. The highest chances of death occur when the LDH level is greater than 1200 IU/L and the D-dimer level is greater than 3 µg/mL.

These markers can help determine how aggressively patients should be treated, or if the patient should be discharged and how to monitor them, among other clinical decisions.

In our study, we did not find statistically significant differences when comparing vitamin D levels with the SARS-CoV-2 antibody index, nor when we tried to correlate ferritin levels with the antibody index, in the 91 patients studied ($p > 0.05$).

Nor have we found a significant correlation ($p > 0.05$), when trying to correlate vitamin levels with serum ferritin levels.

This may be due to the fact that our patients studied were not stratified according to clinical severity and were patients who were in hospitalization and in the ICU and in different phases of the disease.

CONCLUSIONS

The inflammatory storm is frequently found in patients with Covid-19 and the early detection of the state of hyperinflammation through cytokine monitoring may favor an early approach in patients with Covid-19 who will evolve unfavorably. The most useful inflammation markers today are IL-6, D-dimer, ferritin, and vitamin D; and immunosuppressive treatments that counteract the state of hyperinflammation are currently used to minimize the inflammatory storm in these patients.

In our study, we did not find statistically significant differences when comparing vitamin D levels with the SARS-CoV-2 antibody index in the 159 patients studied. Although we have observed in our case, that there is an important group of patients who have the highest levels of Vitamin D, with the lowest levels of antibodies against SARS-CoV-2.

Nor have we found statistically significant differences when comparing ferritin levels with the SARS-CoV-2 antibody index in the 91 patients studied.

When we compared vitamin D levels with ferritin levels, we were also unable to find statistically significant differences in their correlation. This could be due to the fact that our studied patients were not stratified according to clinical severity and were patients who were in hospitalization and in the ICU and in different phases of the disease.

Covid-19 patients with lower vitamin D levels also have elevated serum levels of inflammatory markers such as ferritin and other markers. Therefore, if the treatment with vitamin D plays a role in the prevention of the disease or in the improvement of the prognosis of patients with Covid-19; this will have to be elucidated in large randomized controlled trials, as these studies are necessary to precisely define the role of supplementation with this vitamin in future waves of SARS-CoV-2 infection.

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