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Vitamin D Levels as a Marker of Severe SARS-CoV-2 Infection

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Abstract: Severe infection from the SARS-CoV-2 virus is associated with various clinical findings, including hematological manifestations. Thrombotic events or a tendency to develop thrombotic events also characterize severe COVID-19 disease and may lead to death. Vitamin D is known to have immunomodulating properties and to enhance the body defense system against invading pathogens and to have immunostimulatory properties as far as the innate immune response is concerned. The aim was to measure 25(OH)D$_3$ levels in patients hospitalized for severe COVID-19 infection, to explore the relationship between 25(OH)D$_3$ and outcome and to investigate the relationship between 25(OH)D$_3$ levels, CRP, ferritin, d-dimers and fibrinogen levels in this cohort. In a cohort of 88 patients hospitalized for severe infection from the SARS-CoV-2 virus and a control group matched for age and sex the levels of 25(OH)D$_3$ were measured. In the same cohort CRP, ferritin, d-dimer and fibrinogen levels were also analyzed. Levels of 25(OH)D$_3$ were 17.36±8.80 ng/ml (mean±SD) as compared with 24.34±10.34 ng/ml, in patients with severe SARS-CoV-2 infection and the control group, respectively, $p<0.001$ (Student's t test). The levels of 25(OH)D$_3$ were found to be significantly related to outcome, i.e., survival as opposed to non-survival, as more patients with 25(OH)D$_3$ deficiency (0-10 ng/ml) and insufficiency (10-20 ng/ml) had a fatal outcome as compared with those with vitamin D sufficiency, $p<0.001$, chi-square test, $p<0.001$, Fischer's exact test. Levels of 25(OH)D3 were inversely related to CRP, ferritin, d-dimer and fibrinogen levels, $p<0.001$, linear regression analysis, beta coefficient of variation, -0.176, -0,160, -0.178, -0.158, respectively. Vitamin D deficiency observed in severe SARS-CoV-2 infection was related to the disease outcome. Increased ferritin levels, and increased thrombotic markers, namely d-dimer and fibrinogen levels were observed in the patients and their concentration was also inversely related to vitamin D. We showed that vitamin D levels were low in hospitalized patients with severe SARS-CoV-2 infection and also inversely related to CRP, ferritin, d-dimer and fibrinogen concentration. It is proposed that vitamin D levels may be an index of severity in the context of SARS-CoV-2 infection.

Keywords: vitamin D; SARS-CoV-2; outcome; ferritin; d-dimers; fibrinogen

1. Introduction

The SARS-CoV-2 virus initiated the recent pandemic, probably having originated in the Wuhan region of China [1,2]. The pandemic got the medical profession and society by surprise and no medical treatment was known at the time for the novel viral infection. Therefore, intense research was initiated to combat and prevent the disease leading to the discovery of various agents capable of fighting the infection [3]. In particular, research into the matter of inadequate vitamin D levels which may be related to the severe infection and adverse outcome led to the observation that severe SARS-CoV-2 infection may be related to inadequate vitamin D levels [4–7]. Vitamin D is a hormone related to calcium and bone metabolism with significant immune modulating properties [8]. In particular, it

has been shown that vitamin D may act to enhance the innate immune response [9] and to contribute to the response of the organism to infectious agents, such as mycobacterium tuberculosis [10] and mycobacterium leprae [11]. Vitamin D induces the production of cathelicidin thus having an antiviral effect [12]. Consequently, vitamin D administration was implemented in the management of SARS-CoV-2 infection [13–15].

Dexamethasone was applied as first and second line treatment and led to the discovery that dexamethasone may prevent severe disease and may improve adverse outcome in the form of hospitalization within the acute care unit as well as intubation [16,17]. Infection from the SARS-CoV-2 virus may manifest as a mild viral illness or may take the form of a severe disease which may lead to adverse outcomes [18]. In particular, it may necessitate hospitalization or admission to the acute care unit or it may lead to intubation and in severe cases to death [19].

Thrombosis as well as a tendency to develop thrombosis may accompany a viral infection [20] and it has been reported in relationship to Covid-19 infection [21,22]. In particular, Covid-19 has been proposed to be a thrombogenic virus [23,24] and to cause thromboembolic disease and cardiac thrombotic complications [25]. In accordance, microthrombi have been observed in the lungs at autopsy in patients having died from the SARS-CoV-2 infection [26]. Thrombotic markers, such as fibrinogen and d-dimers have been shown to increase during Covid-19 infection [27,28]. D-dimers may be a predictive factor in identifying thrombosis and high levels may be an index of severity of the infection and death [23]. Anticoagulation is necessary and heparin is considered the preferred anticoagulant as it may have antiviral action [23]. In the paper herein we measured d-dimer and fibrinogen levels and we found increased d-dimer and fibrinogen levels which were inversely related to vitamin D levels. In accordance low vitamin D levels have been associated with thromboembolic events [29] and vitamin D may have an antithrombotic action [30].

Ferritin ensures the availability of iron for cellular activities while protecting the DNA and proteins from the possible toxic effects of iron [31]. Elevated levels of ferritin are observed in infections [32] and are thought to be an inflammatory index and appear to be an important host defense mechanism against bacteria as iron may be cytotoxic to bacteria [33]. Ferritin levels were shown to increase during the course of SARS-CoV-2 infection and the role of ferritin in the course of the disease was discussed [28,34]. In particular, increased ferritin levels may be an index of severity as well as a prognostic factor in SARS-CoV-2 patients [35]. In our study, we observed high ferritin levels in patients with Covid-19 disease, which were inversely related to vitamin D levels.

The aim was to describe the relationship of vitamin D levels with the outcome of the disease in a cohort of patients who were hospitalized for severe SARS-CoV-2 infection. Low vitamin D levels were observed in patients with severe SARS-CoV-2 infection which were related to the outcome of the infection. Vitamin D levels were inversely related to d-dimer, fibrinogen and ferritin levels.

2. Results

The levels of $25(OH)D_3$ were 17.36 ± 8.80 ng/ml (mean±SD) as compared with 24.34 ± 10.34 ng/ml in the control group, $p<0.001$ (Student's t test) (Figure 1). The levels of $25(OH)D_3$ were found to be related to the outcome, i.e., survival as opposed to non-survival, as more patients with $25(OH)D_3$ deficiency (0-10 ng/ml) and insufficiency (10-20 ng/ml) had a fatal outcome as compared with those with $25(OH)D_3$ sufficiency (>20 ng/ml), $p<0.001$, chi-square test, $p<0.001$, Fischer's exact test (Figure 2).
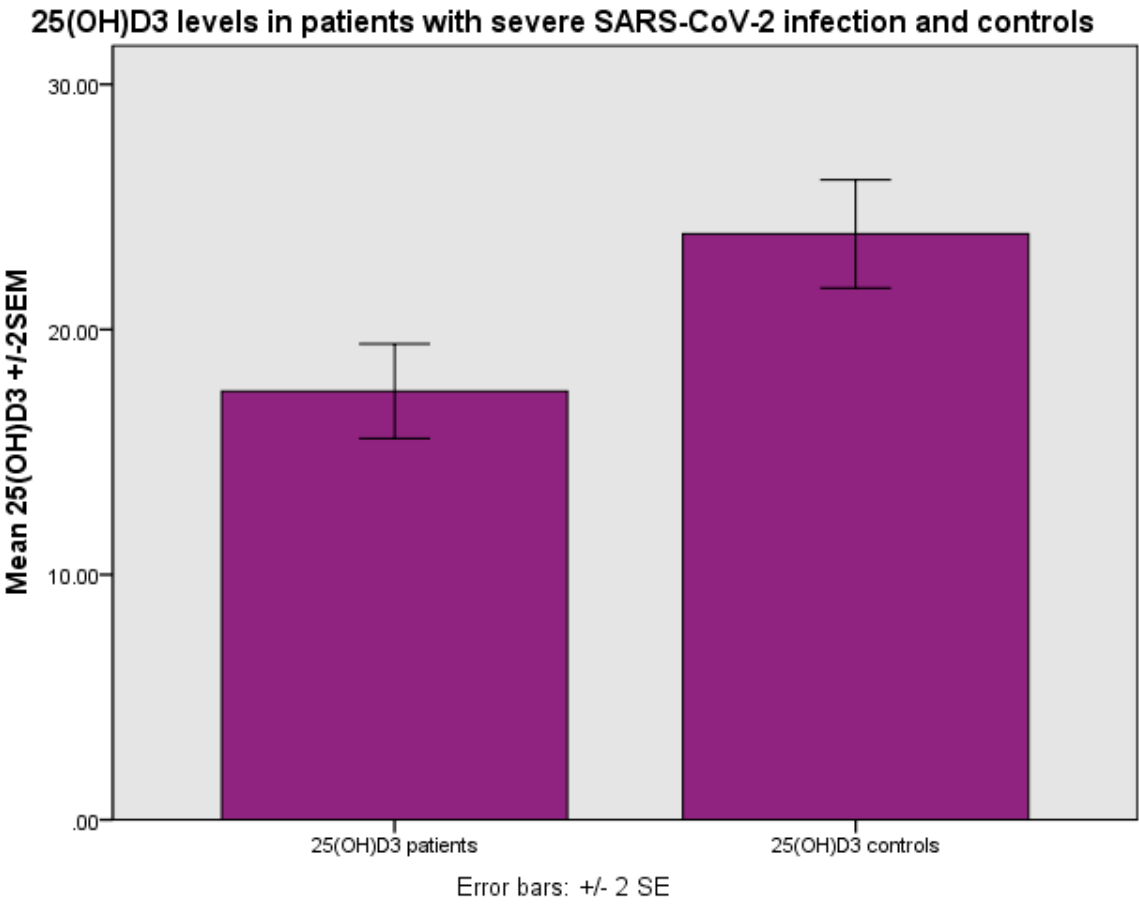
Figure 1. The levels of 25(OH)D$_3$ (ng/ml) in patients with severe SARS-CoV-2 infection and controls, p<0.001 (Student's test).
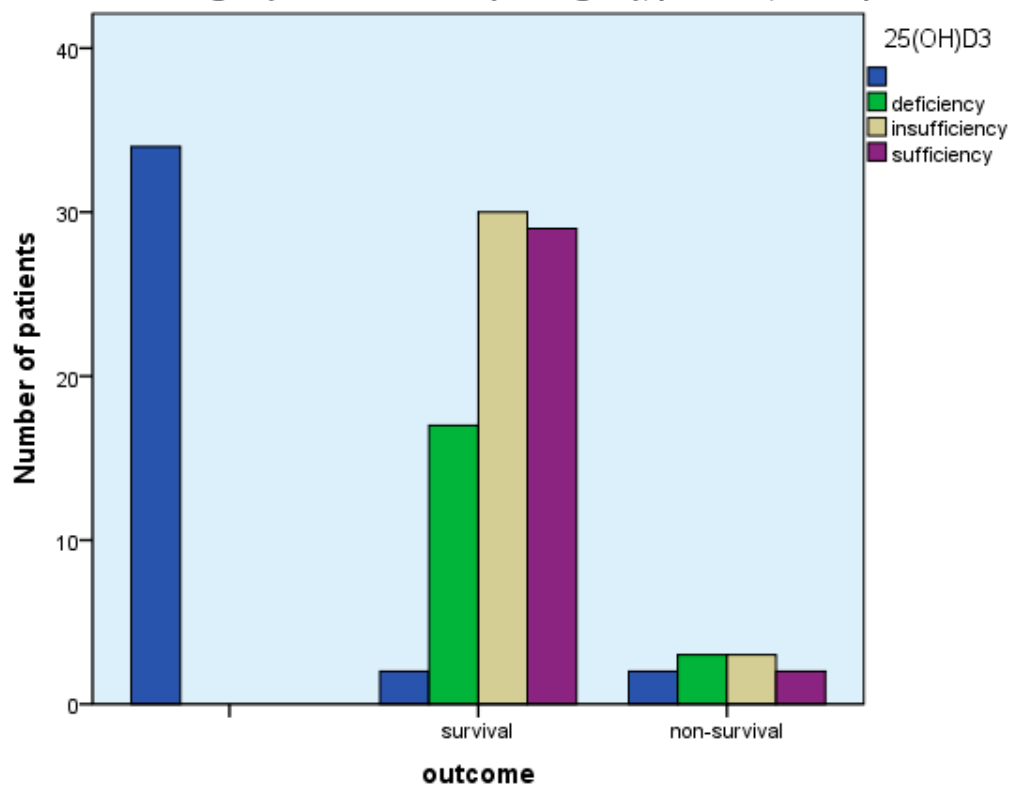
Figure 2. The number of patients who survived and did not survive after severe SARS-CoV-2 infection in relationship to 25(OH)D$_3$ concentration, deficient (0-10 ng/ml), insufficient (10-20 ng/ml) and sufficient (>20 ng/ml), p<0.001, chi square test, p<0.001, Fisher's exact test.

Increased CRP and ESR levels were observed in the SARS-CoV-2 patients. The concentration of 25(OH)D$_3$ was found to be inversely related to CRP, p<0.001, linear regression analysis, standardized coefficient of variation beta -0.176 (Figure 3).

Figure 3. The levels of 25(OH)D$_3$ (ng/ml) in relationship to CRP (mg/L) in patients with severe SARS-CoV-2 infection, linear regression analysis, p<0.001.

The levels of 25(OH)D$_3$ were also found to be inversely related to ferritin, p<0.001, linear regression analysis, standardized coefficient of variation beta -0.160 (Figure 4). Ferritin (ng/ml) levels were found to be related to CRP, p<0.001, linear regression analysis, standardized coefficient of variation beta 0.484 (Figure 5).

Figure 4. The levels of 25(OH)D3 (ng/ml) in relationship to ferritin concentration (ng/ml) in patients with severe SARS-CoV-2 infection, linear regression analysis, p<0.001.
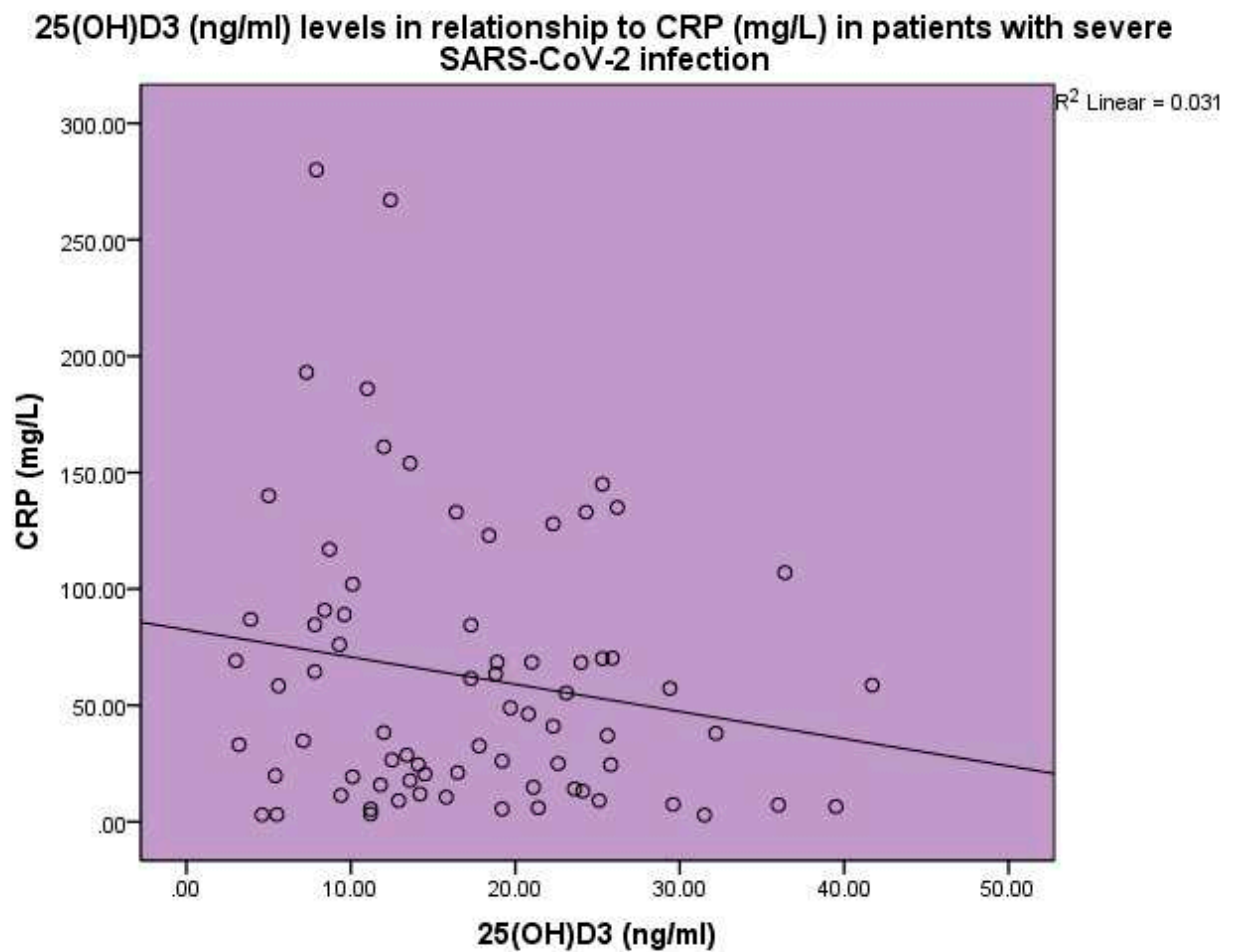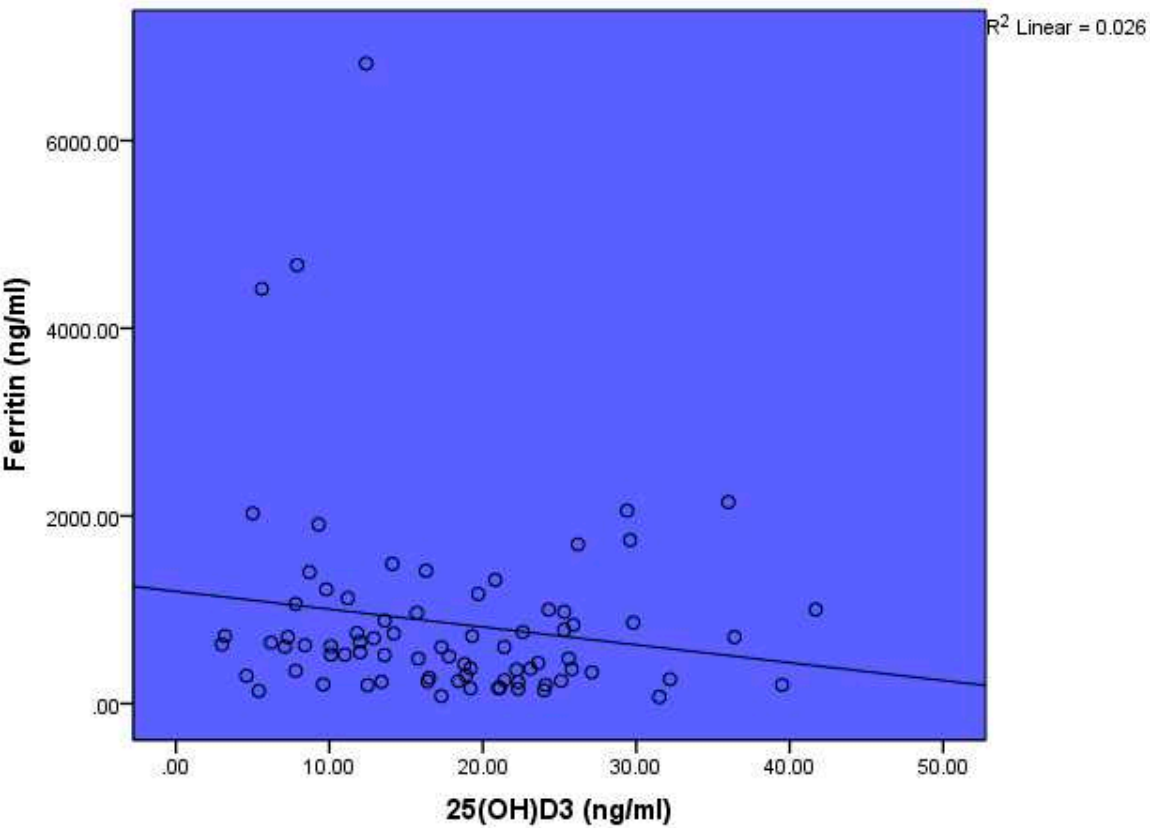
Figure 5. Levels of ferritin (ng/ml) in relationship to CRP (mg/L) in patients with severe SARS-CoV-2 infection, linear regression analysis, $p < 0.001$.

Thrombotic markers were found to be increased in the blood of the sick patients as compared to the expected normal levels. Levels of 25(OH)D$_3$ were found to be inversely related to d-dimer levels, $p < 0.001$, linear regression analysis, standardized coefficient of variation beta -0.178 (Figure 6). Levels of 25(OH)D$_3$ were found to be inversely related to fibrinogen levels, $p < 0.001$, linear regression analysis, standardized coefficient of variation beta -0.158 (Figure 7). Thyroid hormone levels were within normal range in the Covid 19 group of patients. In particular, TSH, FT$_4$ and FT$_3$ levels were within normal range.

Figure 6. The levels of 25(OH)D₃ (ng/ml) in relationship to d-dimers (µg/L) in patients with severe SARS-CoV-2 infection, linear regression analysis, p<0.001.
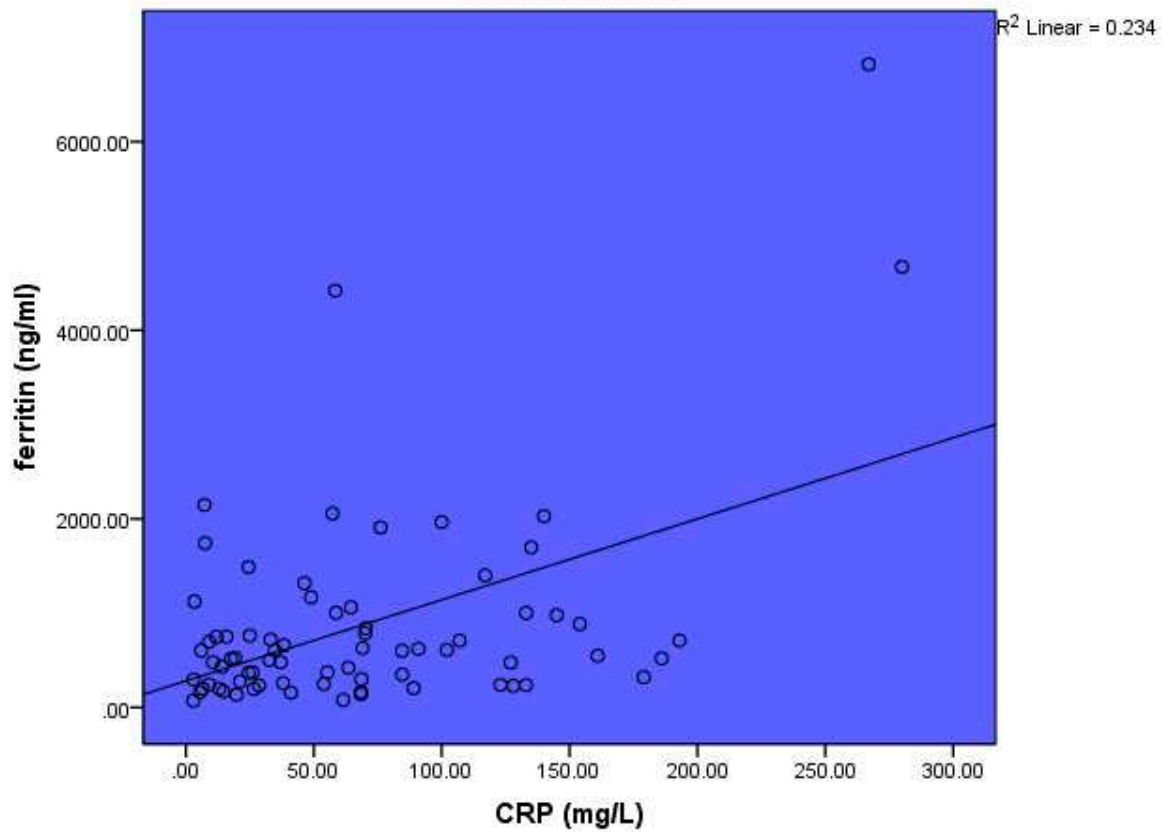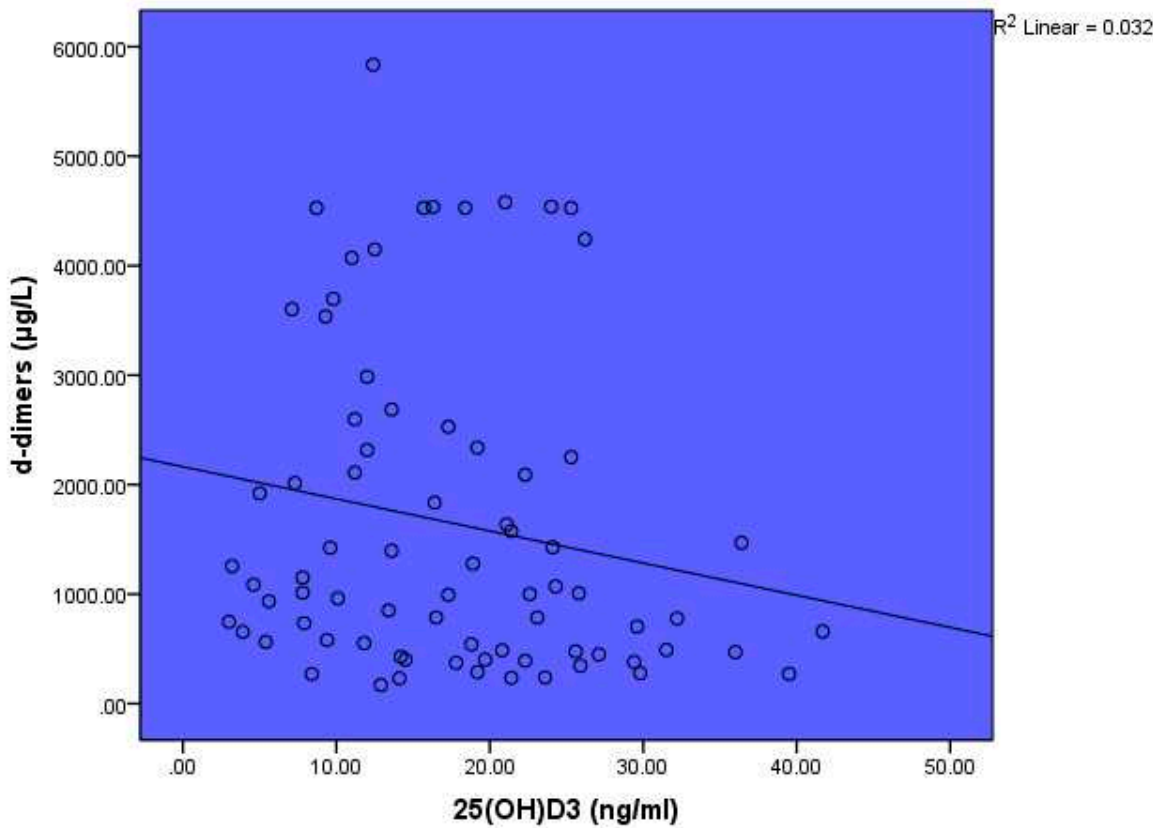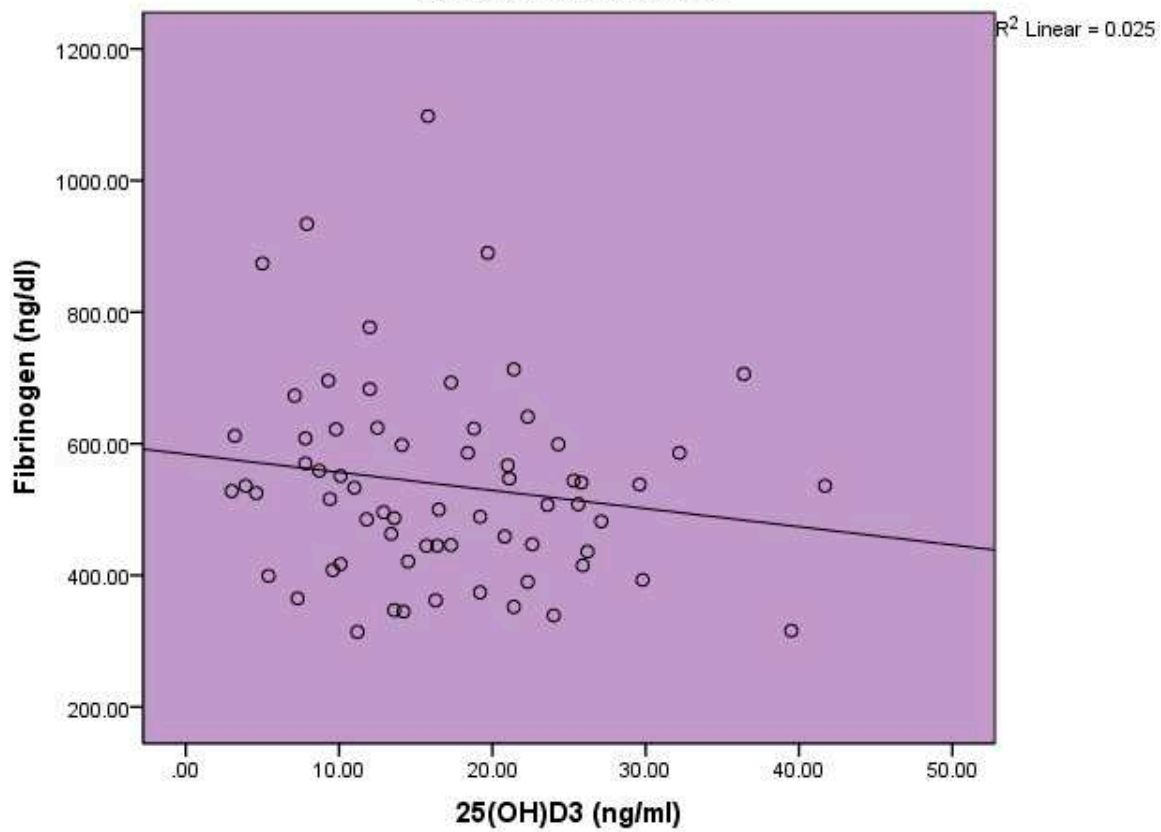
Figure 7. The levels of 25(OH)D$_3$ (ng/ml) in relationship to fibrinogen (ng/dl) in patients with severe SARS-CoV-2 infection, linear regression analysis, p<0.001.

3. Discussion

The SARS-CoV-2 infection may lead to a severe acute disease which necessitates hospitalization and may lead to intubation and death [36,37]. Various agents have been employed to combat the SARS-CoV-2 infection, amongst those vaccines and antiviral medications. As, technology was quick to employ the proper vaccines to combat the COVID-19 pandemic [38,39], antiviral medications were subsequently discovered [40–42] and the use of various agents which might aid in fighting the disease gained the attention of the scientific community. Dexamethasone was found to improve the course of the disease and outcome. Vitamin D when insufficient may lead to a worse outcome, whereas, vitamin D administration may improve outcome [13–15]. In the present study vitamin D insufficiency was observed in SARS-CoV-2 patients and it was found to be related to disease outcome.

Vitamin D deficiency has been observed in patients with severe SARS-C0V-2 infection [4,43–50]. Bassatne et al. [44] in a meta-analysis of 31 peer-reviewed observational studies found a positive trend between levels of 25(OH)D$_3$ <20 ng/ml and an increased risk of mortality, ICU admission, invasive ventilation and non-invasive ventilation. Chiodini et al. [4] performed a meta-analysis of 54 studies on SARS-CoV-2 infection and vitamin D and they found that severe deficiency, deficiency and insufficiency of vitamin D was related to admission to the intensive care unit, mortality, COVID-19 infection and hospitalization. They concluded that patients with vitamin D deficiency present with an increased risk for the development of acute respiratory distress syndrome requiring admission to the intensive care unit, higher mortality and an increased susceptibility to SARS-CoV-2 infection and hospitalization. In our study we observed low vitamin D levels in patients with severe SARS-CoV-2 infection and a relationship between vitamin D deficiency and insufficiency with the outcome, i.e., fatal outcome as opposed to survival. Vitamin D is an immunomodulatory hormone which acts in a

pleiotropic way by both enhancing innate immunity and the response of the organism against various infections and by suppressing the hyperinflammatory response to the viral insult [51]. Vitamin D has been shown to contribute to the response to various pathogens [11,52], especially those attacking the respiratory system [53]. Thus, vitamin D may enhance the innate immune response against the virus and may dampen the hyperinflammatory reaction against the COVID-19 virus [51]. Vitamin D enhances the innate immune response against the SARS-CoV-2 virus by activating toll-like receptor 2 and by peptide synthesis, which act to combat infectious invasions [54]. Vitamin D attenuates the release of proinflammatory cytokines by CD4+ lymphocytes thus inhibiting the development of a cytokine storm [55]. Additionally, vitamin D has been shown to increase the bioavailability and expression of ACE2 [56], which acts as a receptor for the virus and may contribute to the trapping and inactivation of the virus [57].

Vitamin D supplementation has been proposed to play a significant role in the protection of the human organism against the COVID-19 infection. The effect of vitamin D supplementation on COVID-19 prognosis in high-risk older patients was tested in the COVIT-TRIAL, a randomized controlled trial [58]. In the COVIT-TRIAL [59], a multicenter, randomized, controlled, open-label study, performed in France, which involved collaboration of several medical centers patients were randomly allocated to either a single oral high-dose (400,000 IU) or standard-dose (50,000 IU) cholecalciferol administered under medical supervision within 72 hours after the diagnosis of COVID-19. The primary outcome was 14 day mortality from any cause. A group of 254 patients with a median age of 88 years met eligibility critera and formed the intention to treat group. Overall, 8 (6%) of 127 patients allocated to high-dose cholecalciferol, and 14 (11%) of 127 patients allocated to standard-dose cholecalciferol died within 14 days, p = 0.049. However, the protective effect of the single oral high-dose administration was not sustained at 28 days, not leading though to more frequent adverse effects as compared to the standard vitamin D dose. Bilezikian et al. [60] suggested that vitamin D administration should be individualized, according to the needs of the patients and the existing comorbidities, such as kidney and liver disease, which may affect vitamin D metabolism.

Vitamin D levels were found to be inversely related to CRP. This finding has been previously observed [61] in patients with autoimmunity and vitamin D deficiency [62] and in patients hospitalized in the acute care unit [61]. Thus, vitamin D may be a reverse acute phase reactant or a reverse index of the acute phase response [63]. Ferritin levels were found to be increased as opposed to the expected normal range in patients with severe SARS-CoV-2 disease in this study and a negative association was observed between vitamin D and ferritin levels. Ferritin has been found to be increased in patients with COVID-19 disease and its levels were found to be related to disease severity and disease outcome. In a meta-analysis involving 52 studies the authors [34] observed higher ferritin levels in patients with severe COVID-19 infection as compared to patients with non-severe infection. COVID-19 has been included in the hyperferritinemic syndromes [64]. Hyperferritinemia in critically ill COVID-19 patients may be an index of severity as well as a pathogenic mediator [65]. Ferritin in the context of severe COVID-19 disease may act as a possible enhancer of the cytokine storm and may be involved in a vicious pathogenic loop [35,66,67].   In a retrospective cohort study, the association between serum levels of ferritin, soluble CD163, and IL-18 and their impact on the prognosis of COVID-19 was investigated [67]. Data of 70 hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were analyzed. Soluble CD163, ferritin, and IL-18 were measured and the correlation of these parameters with the respiratory deterioration and overall 30-day survival was assessed. A group of 60 patients survived 30 days from hospitalization. There were substantial differences between the subjects who were alive following 30 days compared to those who expired. Higher levels of IL-18 and ferritin were observed in the group with the fatal outcome as compared to those who survived. Soluble CD163, ferritin, and IL-18 were found to correlate with the severity of COVID-19 infection. It was suggested that measuring ferritin and IL-18 during the disease progression may help target them at the right time and refine the decision-making regarding the necessity for hospitalization [67]. As found in our study a relationship has been observed between CRP and ferritin as both are increased during an infection.

Viral infections are related with a tendency to develop thrombosis [68]. The SARS-CoV-2 infection is related with the development of both arterial and venous thrombosis [69]. In an autopsy study of lungs obtained from patients who died from COVID-19 histologic analysis showed widespread thrombosis with microangiopathy [26]. Alveolar capillary microthrombi were prevalent in patients who died from COVID-19. In patients with SARS-CoV-2 infection viral effects, the increase in the vasoconstrictor angiotensin II, the decrease in the vasodilator angiotensin and the release of cytokines in the context of sepsis may lead to coagulopathy. Coagulopathy may be observed in as many as 50% of patients with severe COVID-19 disease.

Studies have shown an increase in d-dimer and fibrinogen levels in patients with COVID-19 disease during the course of the illness [70]. A 3 to 4 fold rise in d-dimer levels may be associated with a poor prognosis [70]. In a meta-analysis of 29 studies [71] it was observed that in patients with SARS-CoV-2 infection increased d-dimer levels on admission were related to an increased risk of disease severity and mortality. In our study increased levels of d-dimer and fibrinogen levels were observed in severe SARS-CoV-2 infection as compared with healthy controls. D-dimer and fibrinogen levels were inversely related to the $25(OH)D_3$ levels. In a small study short-term high dose vitamin D supplementation induced a significant decrease in fibrinogen concentrations, without however an effect on CRP, d-dimer or ferritin, leading to the conclusion that vitamin D might have an antithrombotic effect [72].

In conclusion, lower vitamin D levels were observed in patients with severe SARS-CoV-2 infection as compared to control subjects. Vitamin D deficiency and insufficiency was related to disease outcome. Vitamin D levels were inversely related to CRP, ferritin, d-dimer and fibrinogen levels.

4. Materials and Methods

In a cohort of 88 patients, 47 male, 41 female, hospitalized in the COVID-19 Department over a period of 12 months CRP levels, ESR levels, hemoglobin levels, white blood cell count, ferritin levels, fibrinogen levels, prothrombin levels, d-dimer levels and $25(OH)D_3$ levels were measured (Table 1). The levels of $25(OH)D_3$ were estimated upon admission to the COVID-19 unit. The levels of $25(OH)D_3$ were also measured in a cohort of 88 control subjects matched for age and sex. Patients on treatment with calcium and vitamin D supplements were excluded from the study.

Table 1. Characteristics of patients hospitalized for severe SARS-CoV-2 infection.

	Normal range	Minimum	Maximum	Mean	Std. Deviation
Age (years)		21.0	97.0	67.7	16.9
Sex (male/female)	47/41				
White blood cell count (cells/µL)	4.6-10.2	.71	17300.0	6259.6	3373.5
Neutrophils (%)		37.6	89.9	70.5	13.1
CRP (mg/L)	<5.0	2.9	280.0	65.1	60.6
ESR (mm/1h)	<15	15.0	108.0	44.6	24.4
$25(OH)D_3$ (ng/ml)		3.0	41.7	17.4	8.8
Ferritin (ng/ml)	20-300	71.0	6821.0	851.9	1032.6
d-dimers (µg/L)	0-550	170.0	5835.0	1690.9	1479.5
Fibrinogen (ng/dl)	180-350	314.0	1098.0	543.1	157.2
TSH (µIU/ml)	0.35-4.94	.36	3.36	1.17	.78
FT_3 (pg/ml)	1.58-3.91	2.2	5.1	3.7	.66
K (mmol/L)	3.5-5.2	2.2	5.1	3.7	.6
PT (sec)		10.0	14.3	11.7	1.0
APTT (sec)	26-39	20.9	59.3	31.1	5.1
INR		.86	1.29		.1

25(OH)D$_3$ levels were measured by a 1-step delayed chemiluminenescent microparticle immunoassay (Abbott Park IL) [73]. It is a fully automated immunoassay offered by Abbott on the ARCHITECT platform with a within run CV of 3.0-5.4% and a between run CV of 4.7-6.3%.

CRP was analyzed by particle enhanced immunonephelometry according to the manufacturer's instructions (CardioPhase hsCRP; Siemens Healthcare Diagnostics, Camberley, UK). Polystyrene particles coated with monoclonal antibodies specific to human CRP were aggregated when mixed with samples containing CRP, using equipment provided by Elite Medical, Marietta, GA, USA. These aggregates scattered a beam of light passed through the sample. The intensity of the scattered light was proportional to the concentration of CRP in the sample. The result was evaluated as compared with a standard of known concentration. The sensitivity of the assay was 0.175 mg/L with a coefficient of variation of 7.6% at 0.41 mg/L. No cross-reactivity is known for the assay. The intra-assay coefficient of variation ranged from 2.7% at 14 mg/L to 4.6% at 5.95 mg/L. The inter-assay coefficient of variation ranged from 2.0% at 14 mg/L to 4.0% at 5.95 mg/L.

TSH levels were measured in serum by the ARCHITECT TSH immunoassay (Abbott Park IL) which is a chemiluminescent microparticle immunoassay with an analytical sensitivity of <0.0025 µIU/ml, a precision of <10% and an interassay coefficient of variation of <20%. The ARCHITECT TSH assay is a two-step immunoassy which utilizes chemiluminescent microparticel immunoassay technology with flexible assay protocols, that are referred to as Chemiflex. In the first step, sample, anti-β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent were combined. TSH present in the sample was bound to the anti-TSH antibody coated microparticles. After washing, anti-α TSH acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units. A direct relationship exists between the amount of TSH in the sample and the relative light units detected by the ARCHITECT i optical system.

Free T$_3$ (FT$_3$) levels were measured by the ARCHITECT FT3 assay which is a chemiluminescent microparticle immunoassay (Abbott Park IL), with an analytical sensitivity of <1.0 pg/ml and an analytical specificity of <0.001%. The ARCHITECT Free T3 assay is a two-step immunoassay for the determination of free T$_3$ in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols. In the first step, sample and anti-T3 coated paramagnetic microparticles were combined. Free T$_3$ (unbound) in the sample binded to the anti-T3 coated microparticles. After washing, T$_3$ acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units. An inverse relationship existed between the amount of FT$_3$ in the sample and the relative light units detected by the ARCHITECT i optical system.

Free T$_4$ (FT$_4$) levels were measured by the ARCHITECT FT4 assay which is a chemiluminescent microparticle immunoassay (Abbott Park IL) with an analytical sensitivity of <0.4 ng/dl and a precision of <10%. The ARCHITECT Free T4 assay is a two-step immunoassay to determine the presence of free thyroxine (Free T$_4$) in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols. In the first step, sample and anti-T$_4$ coated paramagnetic microparticles were combined. FT$_4$ (unbound) present in the sample binded to the anti-T$_4$ coated microparticles. After washing, T$_4$ acridinium labeled conjugate was added in the second step. Pre-Trigger and Trigger Solutions were then added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units. An inverse relationship exists between the amount of Free T$_4$ in the sample and the relative light units detected by the ARCHITECT i optical system.

Ferritin levels were analyzed by the Atellica IM Fer assay (Siemens Healthineers), which is a 2-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of 2 anti-ferritin antibodies with an analytical sensitivity of $\leq$0.5 ng/ml and a limit of detection of $\leq$1.0 ng/ml. The Atellica IM Fer Assay utilizes 2 antiferritin antibodies, the first antibody is a goat polyclonal anti-ferritin antibody labeled with acridium ester and the second antibody is a mouse monoclonal anti-ferritin antibody, which is covalently coupled to paramagnetic particles. The

measurement of ferritin was based on the direct relationship which exists between the amount of ferritin present in the sample and the amount of relative light units detected by the system.

D-dimer levels were studied by the Innovance D-Dimer immunoturbidimetric assay (Siemes Healthlineers) with a sensitivity of <0.5 mg/L and a precision, CV% between 5.9-8.4%. The age-adjusted sensitivity of the Innovance D-Dimer immunoturbidometric assay was 98.9% (CI 95%) and the age-adjusted specificity was 77.4% (CI 95%).

Fibrinogen levels were determined by the Multifibren U assay (Siemens Healthineers) which uses a modification of the Clauss method, with a precision, within series CV% of 2.9-7.2%. The Clauss fibrinogen assay is a quantitative, clot-based, functional assay. The assay measures the ability of fibrinogen to form fibrin clot after being exposed to a high concentration of purified thrombin. Multifibren U is an in vitro diagnostic reagent for the quantitative determination of fibrinogen in human sodium citrated plasma by means of automated and manual coagulometric methods. Fibrinogen determination by Multifibren U is standardized against the reference methods by RatnoffMenzie and Kjeldahl [74]. Multifibren U utilizes a modification of the Clauss method. Citrated plasma was brought to coagulation by a large excess of thrombin. The coagulation time was dependent largely on the fibrinogen content of the specimen.

For the evaluation of the results of the present study $25(OH)D_3$ as far as disease outcome (survival versus non-survival) was concerned, the levels of $25(OH)D_3$ were classified as deficiency (0-10 ng/ml), insufficiency (10-20 ng/ml) and sufficiency >20 ng/ml.

The study was approved by the ethical committee of Asclepeion Hospital (approval number 15335, 24/11/2020). Informed consent was obtained from the patients involved in the study.

Statistical evaluation of the results was performed using the SPSS statistical package (IBM SPSS Statistics v27). Data were presented as mean ± SD. Student's *t*-test was used to compare the patient group with the control group. Regression analysis was performed to analyse the relationship between $25(OH)D_3$ levels, CRP, ferritin, d-dimer and fibrinogen levels. Statistical significance was set at a p value of <0.05.

5. Conclusions

In conclusion, low vitamin D levels were observed in patients with severe SARS-CoV-2 infection which were related to the disease outcome and inversely related to ferritin, d-dimer and fibrinogen levels.

Author contributions: Conceptualization, L.A., I.K.A., S.N., A.K.; methodology, L.A, I.K.A, S.N., A.K..; software, O.M.; validation, I.K.A., O.M., P.A. and Y.S.; formal analysis L.A., I.K.A., P.A.; investigation, L.A, S.N., A.K., E.S., C.S..; resources O.M., E.S., C.S..; data curation, L.A., I.K.A., E.S., C.S.; writing—original draft preparation, L.A., I.K.A, Y.S.; writing—review and editing, P.A., Y.S.; visualization, I.K.A., S.N., A.K.; supervision, Y.S.; project administration, I.K.A., P.A., Y.S.; funding acquisition, P.A. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Asclepeion Hospital, Voula, Athens, Greece, approval number 15335, 24/11/2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

Conflicts of Interest: The authors declare no conflict of interest.

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