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Vitamin D in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with non-invasive ventilation support.

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Abstract

Background. Vitamin D (VitD) deficiency has been reported to be associated with respiratory tract infection. In this work we evaluated the concentration of VitD in COVID-19 patients experiencing acute respiratory infections of different levels of severity excluding those who underwent invasive respiratory support.

Methods. The levels of serum VitD and C-reactive protein (CRP) were analyzed in 118 consecutive hospitalized COVID-19 patients (74M, 44F), confirmed with rRT-PCR. Of these patients with ventilation support 52 (44.1%) received oxygen via nasal cannula, oxygen mask or an oxygen mask with a reservoir, 48 (40.7%) were on a continuous positive airway pressure device (CPAP) and 18 (15.3%) on non-invasive mechanical ventilation (NIMV).

Results. The median values (range) of VitD and of CRP were 15.1 ng/mL (1.3-73.3) and 14.2 mg/L (5.0-151.2), respectively. A negative correlation from VitD levels and those of CRP (correlation coefficient – 0.259; $P=0.005$) was observed. VitD levels in O₂ support patients were significantly higher than in both CPAP and NIMV patients. No statistical differences were found for CRP levels ($P=0.834$) among the three type of oxygen support. Fewer patients with O₂ support had VitD <30 ng/mL and <20 ng/mL than CPAP and NIMV patients. There were no relationships between VitD and the three classes of IgM ($P=0.419$) and of IgG ($P=0.862$) SARS-CoV-2 antibodies values. The behavior was the same for CRP.

Conclusion. Our study shows that a significant proportion of COVID-19 patients have a VitD deficiency and that this condition is more frequent in CPAP and in NIMV patients.

Introduction

This new coronavirus infectious disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported on December 1, 2019 and identified as a previously unknown betacoronavirus [1,2]. The pathogen is transmitted among humans through aerosol [3,4] with transmission capability even before symptom onset [5-7]. The clinical manifestations of SARS-CoV-2-infected patients ranged from mild non-specific symptoms to severe pneumonia with organ function damage. Patients did not necessarily have fever at onset, some patients developed after hospitalization [8], and few severe patients even did not have fever.

Serum 25-hydroxyvitamin D (VitD) is considered the best indicator of overall vitamin status because reflects the level of the substrate to the regulation of the active form 1,25(OH)₂D, named calcitriol. In general, the actions of VitD are to help regulate serum calcium concentration, which it does in a feedback loop with parathyroid hormone [9]. Recently, much attention has been paid to the role of VitD in host defense against infection. A large number of well-established data showed antiviral effects of VitD which not only can interfere directly with viral replication, but also can act in immunomodulatory and anti-inflammatory way [10]. It was suggested that the supplementation with VitD and vitamin E might increase resistance to SARS-CoV-2 [11]. Moreover, some studies suggest the effectiveness of VitD as an adjuvant therapy along with antiretroviral agents in HIV-infected patients [12].

C-Reactive Protein (CRP) is a well-established biomarker in many clinical settings, but its specificity has been traditionally considered not enough to be a useful guide in the diagnosis of pneumonia. In fact, virtually all infective, autoimmune, ischemic and neoplastic diseases can increase serum CRP values. Nevertheless, some studies have confirmed that it may have a good performance in defining pneumonia severity [13,14]. CRP increases during COVID-19 infection, and higher concentrations are often related to the severity of the disease [15].

For all these reasons we evaluated both the VitD and the CRP levels in a group of COVID-19 patients experiencing an acute respiratory infection to assess the VitD concentration among patients with three

kinds of ventilation supports in the pulmonary complications such as oxygen (via nasal cannula, oxygen mask or oxygen mask with a reservoir), continuous positive airway pressure device (CPAP) and non-invasive mechanical ventilation (NIMV) and for understanding future containment strategies.

Methods

The study was carried out in the Respiratory Unit, ASST Santi Paolo e Carlo, San Paolo Hospital, Milan, Italy and it was approved by local Ethical Committee Milano Area 1 (n. 2020/ST/057) with a priori patient or appropriate proxy consent obtained prior to the participants' entry into the study which was then carried out in accordance with the Helsinki Declaration of the World Medical Association.

The inclusion criteria were age equal to or greater than 18 years and novel coronavirus infection confirmed by real-time polymerase chain reaction according to the World Health Organization (WHO) interim guidelines [16] plus chest computed tomography demonstrating lung involvement. Both genders were included.

Serum samples were obtained from all subjects at their initial observation and kept frozen at -20°C until analysis.

Patients

From April 8 to May 25 2020, 118 consecutive patients (74 males, 44 females; median age 61.0 years, range 24-92 years) met these criteria and were enrolled in the study.

Four patients (3.4%) were seen as outpatients because having minimal clinical signs, 55 were hospitalized at the time of the study (46.6%), 53 were discharged (44.9%) and 9 (7.6%) had died.

Novel Coronavirus (SARS-CoV-2) antibodies were also evaluated using the commercially available kit BioMedomix IgM-IgG Combined Antibody Rapid Test (Morrisville NC, USA). It is one of the world's first rapid point-of-care lateral flow immunoassays for the diagnosis of coronavirus. The test

has been used widely by the Chinese Center for Disease Control to combat COVID-19 infections and is now being made available globally. This IgG-IgM combined antibody test kit, has a sensitivity of 88.66% and a specificity of 90.63% [17].

Based on the results of this assay, 15 patients (12.7%) were negative for IgM, 19 (16.1%) were weakly positive and the remaining 84 (71.2%) were positive at high title; regarding IgG, 12 patients (10,2%) were negative, and 106 (89.8%) were positive at high title.

All patients were treated according to current therapeutic modalities [18]; regarding ventilation support, 52 (44.1%) received oxygen via nasal cannula, oxygen mask or an oxygen mask with a reservoir, 48 (40.7%) were on a CPAP and 18 (15,3%) on NIMV. The three ventilation supports are applied to patients with mild, moderate and severe respiratory distress at presentation.

Serum assays

Serum VitD levels were measured using a chemiluminescence assay (25 OH Vitamin D TOTAL Assay, DiaSorin, Saluggia, Italy). The within-run coefficient of variation (CV) was 3.7-7.7% and the total imprecision CV was 5.8-10.9%; the detection limit of the assay was 4 ng/mL. The assay was carried out using the LIAISON® Analyzer (DiaSorin, Saluggia, Italy). Concentration of VitD <10.0 ng/mL (<25.0 nmol/L) is adopted as “severely deficient”, between 10 to 20 ng/mL as “deficient” and between 20.0 to 30.0 ng/mL as “insufficient” while optimal levels were defined as VitD >30.0 ng/mL [19,20].

Serum CRP levels were measured using commercially available kits (Ortho-Clinical Diagnostics, High Wycombe, United). The assay was carried out using the VITROS® 5600 Integrated System (Ortho-Clinical Diagnostics, High Wycombe, United). The within-run coefficient of variation (CV) was 1.8-2.6% and the total imprecision CV was 3.8-8.4%; the detection limit of the assay was 2.72 mg/L and the reference values < 10 mg/L.

Statistical analysis

No statistical sample size calculation was carried out a priori, and the sample size was equal to the number of patients treated during the study period. Data were reported as median values and range for the continuous variables and as absolute number and percentage for the categorical variables. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the blood parameters and Vit D and CRP were not normally distributed also after logarithm transformation. Statistical analyses were carried out using the Mann-Whitney U-test, the Spearman rank correlation and the chi squared test. The statistical analyses were carried out by running the SPSS/PC+ statistical package (SPSS Inc., ver. 23.0 Chicago, IL, USA) on a personal computer. Two-tailed P values less than 0.05 were considered statistically significant.

Results

There were no differences in age between male (median age 63.0 years, range 24.0-92.0 years) and female (median age 60.0 years, range 39.0-89.0 years) patients at the onset of COVID-19 ($P=0.531$). In the 118 patients of this study, respectively, (Table 1) without significant difference between males and females ($P=0.636$ and $P=0.787$, respectively). Serum levels of VitD were not related to the age of patients (correlation coefficient 0.151; $P=0.103$), whereas those of CRP were related (correlation coefficient 0.211; $P=0.022$). The correlation between the values of VitD and those of CRP is reported in Figure 1 and we found that VitD serum levels were inversely related to those of CRP (correlation coefficient -0.259 ; $P=0.005$). Thirty-four patients (28.8%) had severe VitD deficiency, 53 (44.9%) had deficient levels, 17 (14.4%) had VitD insufficiency and only 14 (11.9%) had optimal levels of VitD (>30 ng/mL). The difference among sex and the various groups of classes of vitamin D were not statistically different ($P=0.551$). In addition, CRP levels >10 mg/L were present in 78 (66.1%) out 118 patients as compared to 40 (33.9%) having CRP less than or equal to 10 mg/L without differences between males and females ($P=0.436$).

There were no differences among the four classes of VitD levels (severe insufficiency, insufficiency, deficient and optimal value) and the type of hospital outcome (0.192) (Table 2); however, 89% of the patients who died had VitD <20 ng/mL. It should be important to note that the percentage of abnormal values of CRP increase from outpatients to those who died ($P=0.005$), and all patient in this latter group had elevated values of CRP at the time of the death (Table 2).

Most important, there were a statistically difference among the classes of VitD and the type of the oxygen support, ($P=0.041$) (Table 3). VitD levels (median and range) in O₂ support patients (18.3 ng/mL, 4.3–73.3) were significantly higher than both CPAP (12.6 ng/mL, 3.8–48.5) and NIMV (15.2 ng/mL, 1.3–31.8) patients; but no difference was observed between the levels of VitD among CPAP and NIMV patients. No statistically differences were found for CRP levels ($P=0.834$) among the three type of oxygen support (Table 3). Furthermore, 80.7% and 57.7% of O₂ support patients, 93.7% and 85.4% of CPAP patients, 94.4% and 85% of NIMV patients had levels of VitD <30 ng/mL and <20 ng/mL, respectively. Then, there was a trend toward lower mean VitD level with increasing acute lung injury, but this was not statistically significant.

As reported in Table 4, there were no relationships when taking into consideration VitD and the three classes of IgM ($P=0.419$) and of IgG ($P=0.862$) novel Coronavirus (SARS-CoV-2) antibodies values (negative, weakly positive, positive at high titre). The behavior is the same for CRP (Table 4).

Discussion

VitD is able to reduce the risk of microbial infection regulating the cellular natural immunity and adaptive immunity [21]. In addition, VitD is able to maintain tight junctions, gap junctions, and adherens junctions [22] and this is probably the mechanism by which prevents the entrance of viruses and bacteria into the human body [23,25]. VitD enhances cellular innate immunity partly through the induction of antimicrobial peptides [26,27], and defensins [28] and also enhances cellular immunity by reducing the cytokine storm induced by the innate immune system, that is the case of COVID-19.

In fact, VitD can reduce the production of pro-inflammatory cytokines [29] and increases the

expression of anti-inflammatory cytokines by macrophages [30]. Regarding HIV, conflicting data have been found. Some authors have described that there is no evidence of higher VitD deficiency in HIV-infected patients compared to non-HIV adults [31]. However, others have reported that VitD deficiency was more prevalent in HIV-positive than in HIV-negative individuals [32].

On 31 December 2019, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, Hubei province, China, and caused the outbreak of the Coronavirus Disease 2019 (COVID-19). We have two methods for the therapy of viral infections; the first is to block viral entry or its replication and the second is to promote persistent cellular and humoral immunity for the uninfected population via vaccination [33]. At present, there are no approved medication to block or limit COVID-19 entry or replication, and vaccine remains to be developed and the work is in progress [33]. From a practical point of view the main problem of COVID-19 is the exaggerated immune-response involving mainly the lung [34] as demonstrated by pathological evaluation. From a molecular point of view the inflammatory cascade initiated by macrophages contributes to both viral control and tissue damage and the production of type I and type III interferons promotes intracellular antiviral defenses in neighboring epithelial cells, which may limit viral dissemination, while release of IL-6 and IL-1 β promotes recruitment of neutrophils and cytotoxic T cells. Within the lung parenchyma, activated neutrophils release leukotrienes and reactive oxygen species that directly induce pneumocyte and endothelial injury, directly leading to acute lung injury. As local viral control is achieved, macrophage-derived IL-6 promotes T follicular helper differentiation as well as B cell germinal center formation and antibody production to confer longterm immunity [35]. In severe or persistent viral infections, however, persistent neutrophil-mediated alveolar damage leads to interstitial flooding, ventilation/perfusion mismatching, and hypoxemic respiratory failure [36]. Several pharmacological substances have been proposed to treat virus complication and their efficacy is under investigation [37]. It has been proposed using VitD [38] to prevent the excessive inflammatory response to infectious diseases by inhibiting the maturation of dendritic cells (DCs) [39]. In contrast, VitD deficiency is associated with greater inflammation (upregulation of CXCL10,

IL-6, TNF- α , and D-dimer) and activated monocyte phenotypes (CX3CR1+ and CCR2+) as found in HIV-infected patients [40-42]. On the other hand, a large number of well-established data showed antiviral effects of vitamin D, which can interfere directly with viral replication, but also can act in an immunomodulatory and anti-inflammatory way [43]. However, the data on vitamin D serum levels in COVID-19 patients are scarce and, for this reason, we undertook the present study.

In this study of 118 COVID-19 patients we found that the mean serum levels of VitD was 17.4 ± 11.4 mg/mL. From a cohort of 107 patients from Switzerland (73 y of median age) were found similar VitD levels, taking count of the older age [44]. It is important to note that 104 (88.1%) of our 118 patients had values of VitD under the optimal level (73.7% deficient and 14.4% insufficient). It has been reported that serum VitD concentrations tend to decrease with age [45]. Then, the possible explanation of our finding is that the major part of our patients were older than 60 years. On the other hand, serum CRP concentrations, 33.0 ± 39.4 mg/L, are related with advanced age and this support the fact which may be important for COVID-19 because case-fatality rates increase with age [46]. Our findings on VitD are very important and confirm the data of a study of Kenny and colleagues who report that in Europe, despite being sunny, Spain and Northern Italy had high rates of VitD deficiency and have experienced some of the highest COVID-19 infection and mortality rates in the world [47]. On the contrary, the northern latitude countries of Norway, Finland and Sweden, have higher VitD levels despite less sunlight exposure, because supplementation and fortification of foods is more common and Nordic countries seems to have lower COVID-19 infection and death rates. However, we found no differences among the four classes of VitD levels (severe insufficiency, insufficiency, deficiency and normal values) and the type of hospital outcome, whereas a statistical differences exist among the classes of vitamin D and the type of the oxygen support. VitD deficient were as percent higher in NIMV (89%) and in CPAP (85%) than in O₂ (57%). Optimal level of the VitD were as percent lower in the same oxygen support groups (5%, 6% and 20%, respectively). The above results show that an association between VitD level and respiratory lesions exists. We know that VitD deficiency may contribute to the pathogenesis of viral infection by negatively modulating

the innate and adaptive immune responses [48] and as a consequence we think that high level of VitD have a role of defense of lungs against the COVID-19. This is confirmed considering that patients needing a less invasive respiratory support, had higher level of VitD. Patients with more severe respiratory support were more frequent in the deficient and severe deficient VitD groups (levels < 20 mg/mL). Additional confirmation of the protective role of VitD can be seen from the negative correlation between VitD and CRP that is a measure of infection severity. In fact, patients with severe disease symptoms have significantly higher CRP levels [49]. A similar negative correlation has been reported in patients with HIV and with pulmonary tuberculosis [50,51]. In addition, a significant increase of CRP at the initial stage, early predicts a severe COVID-19, assessing disease severity and predicting outcome [52,53].

The role of vitamin D in the context of viral infections is also supported by findings of certain VitD receptor gene (VDR) alleles that are associated with increased susceptibility to respiratory infections [54], as well as with the progression of HIV infection [55]. The protective role of vitamin D has been reported in many conditions associated with pneumonia, cytokine hyperproduction, acute respiratory distress syndrome (ARDS) and as a drug for influenza A H5N1 virus-induced lung injury [56,57]. Additionally, some studies suggest the effectiveness of vitamin D as an adjuvant therapy along with antiretroviral agents in HIV-infected patients [58] for their anti-inflammatory and antioxidants properties [59].

Research like this is still exploratory and we need further trials to have concrete evidence on the level of VitD that is needed for optimal immune function. However, low VitD status in the population adds further weight to some sort of mandatory vitamin D fortification policy and recent Irish guidelines suggest that all adults should take at least 400 IU vitamin D daily [60].

In conclusion, we suggest routine measurement of VitD in individuals living in COVID-19 areas, with replacement therapy is required in those who are VitD deficiency. In infected patients VitD may improve immunological recovery during treatment, reduce levels of inflammation and immune activation, and increase immunity against pathogens.

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Table 1. Values of the biochemical assays in the 118 patients studied. Results are reported as median and range. P values are also reported.

	Reference limit	Median value (range)	P value
Vit D	>30 ng/ml	15.1 (1.3-73.3)	
<i>Males (n=70)</i>		15.0 (1.3-73.3)	0.636
<i>Females (n=38)</i>		15.7 (3.5-61.9)	
C-reactive protein	<10 mg/L	14.2 (5.0-151.2)	
<i>Males (n=70)</i>		14.4 (5.0-151.2)	0.787
<i>Females (n=38)</i>		14.2 (5.0-135.5)	

Table 2. Vitamin D and C-reactive protein classes according to the type of hospital stay. Results are reported as absolute number of the case and the relative within group percentage (%).

	Outpatient	Hospitalized	Discharged	Death
Vitamin D				
<i>Severe deficiency</i>	1 (2.9%)	19 (55.9%)	10 (29.4%)	4 (11.8%)
<i>Deficiency</i>	1 (1.9%)	22 (41.5%)	26 (49.1%)	4 (7.5%)
<i>Insufficient</i>	2 (11.8%)	4 (23.5%)	10 (58.8%)	1 (5.9%)
<i>Optimal values</i>	0 (0.0%)	7 (50.0%)	7 (50.0%)	0 (0.0%)
C-reactive protein				
<i>Normal levels</i>	4 (10.0%)	16 (40.0%)	20 (50.0%)	0 (0.0%)
<i>High levels</i>	0 (0.0%)	52 (44.1%)	53 (44.9%)	9 (7.6%)

Table 3. Vitamin D and C-reactive protein classes according to the type of oxygen support. Results are reported as absolute number of the case and the relative within group percentage (%).

	O₂ support	CPAP	NIMV
Vitamin D			
<i>Severe deficiency</i>	13 (38.2%)	16 (47.1%)	5 (14.7%)
<i>Deficiency</i>	17 (32.1%)	25 (47.2%)	11 (20.8%)
<i>Insufficient</i>	12 (70.6%)	4 (23.5%)	1 (5.9%)
<i>Optimal values</i>	10 (71.4%)	3 (21.4%)	1 (7.1%)
C-reactive protein			
<i>Normal levels</i>	18 (45.0%)	15 (37.5%)	7 (17.5%)
<i>High levels</i>	34 (43.6%)	33 (42.3%)	11 (14.1%)

Table 4. Vitamin D and C-reactive protein classes according to the results of IgM and IgG novel Coronavirus (SARS-CoV-2) antibodies (Immunoglobulin [Ig] M/G. Results are reported as absolute number of the case and the relative within group percentage (%).

IGM SARS-CoV-2 antibodies	<i>Negative</i>	<i>Weakly positive</i>	<i>Positive</i>
Vitamin D			
Severe deficiency	5 (14.7%)	8 (23.5%)	21 (61.8%)
Deficiency	7 (13.2%)	7 (13.2%)	39 (73.6%)
Insufficiency	0 (0.0%)	2 (11.8%)	15 (88.2%)
Optimal values	3 (21.4%)	2 (14.3%)	9 (64.3%)
<i>C-reactive protein</i>			
Normal levels	5 (12.5%)	9 (22.5%)	26 (65.0%)
High levels	10 (12.8%)	10 (12.8%)	58 (74.4%)
IGG SARS-CoV-2 antibodies			
Vitamin D			
Severe deficiency	3 (8.8%)	0 (0.0%)	31 (91.2%)
Deficiency	6 (11.3%)	0 (0.0%)	47 (88.7%)
Insufficiency	1 (5.9%)	0 (0.0%)	16 (94.1%)
Optimal values	2 (14.3%)	0 (0.0%)	12 (85.7%)
<i>C-reactive protein</i>			
Normal levels	5 (12.5%)	0 (0.0%)	35 (87.5%)
High levels	7 (9.0%)	0 (0.0%)	71 (91.0%)

Figure legend

Figure 1. Relationship between individual values of Vitamin D and C-reactive protein in 118 patients having COVID-19.

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C-reactive protein	<10 mg/L	14.2 (5.0-151.2)	
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Deficiency	7 (13.2%)	7 (13.2%)	39 (73.6%)
Insufficiency	0 (0.0%)	2 (11.8%)	15 (88.2%)
Optimal values	3 (21.4%)	2 (14.3%)	9 (64.3%)
C-reactive protein			
Normal levels	5 (12.5%)	9 (22.5%)	26 (65.0%)
High levels	10 (12.8%)	10 (12.8%)	58 (74.4%)
IGG SARS-CoV-2 antibodies			
Vitamin D			
Severe deficiency	3 (8.8%)	0 (0.0%)	31 (91.2%)
Deficiency	6 (11.3%)	0 (0.0%)	47 (88.7%)
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Optimal values	2 (14.3%)	0 (0.0%)	12 (85.7%)
C-reactive protein			
Normal levels	5 (12.5%)	0 (0.0%)	35 (87.5%)
High levels	7 (9.0%)	0 (0.0%)	71 (91.0%)

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