Vitamin D deficiency as risk factor for severe COVID-19: a convergence of two pandemics

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Key points

- **Question:** does vitamin D deficiency predispose to severity of SARS-CoV-2 infection?
- **Findings:** in this observational study on 186 consecutive patients hospitalized with PCR-confirmed SARS-CoV-2 infection, we find that patients with severe COVID-19 show lower median serum 25(OH)D and a higher percentage of vitamin D deficiency at intake than a season/age-matched reference population. The correlation between vitamin D deficiency and the need for hospitalization due to COVID-19 was only seen in male patients. In males but not females, the percentage of vitamin D deficient patients also increased with more advanced COVID-19 disease stage as measured by CT.
- **Meaning:** our data indicate a strong statistical correlation between the degree of vitamin D deficiency and severity of COVID-19 lung disease. With more than 1 billion people worldwide affected by vitamin D deficiency, vitamin D supplementation might be a lifesaving, inexpensive, accessible and safe component of primary prevention during the SARS-CoV-2 pandemic and beyond

Structured abstract

Importance: Vitamin D deficiency increases the incidence of respiratory virus infections. More than 1 billion people worldwide are vitamin D deficient. If vitamin D deficiency is associated to incidence or severity of SARS-CoV-2 infection, a global call could be made for vitamin D supplementation to mitigate the pandemic.

Objective: to determine if lower serum 25-hydroxyvitamin D (25(OH)D) levels are correlated to the risk for COVID-19 and its severity as measured by CT

Design: single-center observational study

Setting: AZ Delta general hospital

Participants: 186 consecutive patients with PCR-confirmed SARS-CoV-2 infection hospitalized for COVID-19 from March 1, 2020 to April 7, 2020

Main outcome and measures: comparative analysis of 25(OH)D levels in patients hospitalized for COVID-19 at various radiological stages and a season/age/sex-matched diseased control population

Results: we report on 186 SARS-CoV-2 infected patients requiring hospitalization for severe COVID-19: 109 males (median age 68 years, IQR 53-79 years) and 77 females (median age 71 years, IQR 65-74 years). At admission patients were screened by CT to determine temporal changes of COVID-19 lung disease and classified as stage 1 (ground glass opacities), 2 (crazy paving pattern) and 3 (consolidation). At intake, 25(OH)D levels were measured and compared to a season-matched population of 2717 diseased controls, consisting of 999 males (median age 69 years, IQR 53-81 years) and 1718 females (median age 68 years, IQR 43-83 years). Male and female COVID-19 patients combined showed lower median 25(OH)D than controls (18.6 ng/mL, IQR 12.6-25.3, versus 21.5 ng/mL, IQR 13.9-30.8; P=0.0016) and a higher fraction of vitamin D deficiency (58.6% versus 45.2%, P=0.0005). A strong sexual dimorphism was found: female patients had comparable vitamin D status as control females. Male COVID-19 patients, however, showed markedly higher percentage of vitamin D deficiency than controls (67.0% versus 49.2%, P=0.0006) and this effect was more pronounced with advanced radiological stage ranging from 55.2% in stage 1 to 74% in stage 3.

Conclusions and relevance: vitamin D deficiency is a possible risk factor for severe SARS-CoV-2 infection in males. Vitamin D supplementation might be an inexpensive, accessible and safe mitigation for the SARS-CoV-2 pandemic.

Introduction

In severe SARS-CoV-2 infections, sustained and excessive activity of pro-inflammatory immune cells and cytokines is thought to contribute to alveolar and endothelial damage, triggering a vicious cycle that evolves towards severe COVID-19 with diffuse alveolar damage [1,2]. Beside its role in calcium metabolism, 1,25-dihydroxyvitamin D (25(OH)D) is a pleiotropic regulator of the immune system [3,4]. It stimulates the expression of cathelicidins and beta-defensin in respiratory epithelia as barrier to pathogen invasion [5,6]. Vitamin D generally acts as a pro-survival hormone by enhancing pathogen clearance and as pro-tolerogenic cytokine dampening excessive inflammation by inhibiting neutrophils and switching Th1 CD4 T cells and M1-polarized macrophages towards a type II immunity. Vitamin D deficiency increases the severity of respiratory virus infections [7,8] and contributes to variations in their incidence across seasons, age groups, socioeconomic status and geographies. With up to a third of the world population affected by vitamin D deficiency [9], we studied if 25-dihydroxyvitamin D (25(OH)D) levels influence the risk for COVID-19 and are correlated to its radiological stage in a cohort of 186 patients admitted to a large Belgian community hospital.

Methods

Patients This is a retrospective observational study on 186 consecutive patients hospitalized for COVID-19 pneumonia from March 1, 2020 to April 7 at AZ Delta General Hospital in Roeselare, Belgium. The study was approved by the local ethical committee (AZ Delta Institutional Review Board, IRB number pending) with a waiver of informed consent from study participants considering the study is based on secondary analysis of existing data and poses no risks to the subjects.

Procedures On admission, all patients received a thorax CT (Detailed CT scanning protocol in Supplementary Information) to determine the disease stage by consensus evaluation of the predominant radiological presentation: ground-glass opacities (early stage, 0-4 days, “stage 1”), crazy paving pattern (progressive stage, 5-8 days, “stage 2”), (3) consolidation (peak stage, 10-13 days, “stage 3”) [10]. SARS-CoV-2 infection was confirmed in all patients by PCR for E/N/RdRP genes (Allplex™ 2019-nCoV assay, Seegene, Korea) on nasopharyngeal swab. 25(OH)D was measured on

admission by Elecsys® vitamin D total II traced to the official reference ID-LC-MS/MS (Roche, Switzerland) and referenced to levels in a diseased control population (1718 females, 999 males, aged >18 years Table 1) measured in the same laboratory during the same seasonal period from February 15, 2019 to April 15, 2019.

Statistical analysis Data (not normally distributed) are expressed as medians (IQR) and Mann-Whitney test was used to test statistical differences between groups. Proportions for categorical variables were compared using chi-squared test. Statistical analyses were performed using MedCalc (version 12.2.1, Belgium) and considered significant if P value was less than .05 (Exact P values listed in Supplementary Information Table S1).

Results

Demographics and COVID-19 disease staging. A total of 186 SARS-CoV-2 infected patients were admitted for COVID-19 pneumonia: 109 males (median age 68 years, IQR 53-79 years) and 77 females (median age 71 years, IQR 65-74 years). On admission, patients were screened by CT to determine temporal changes of COVID-19 lung disease and classified based on the predominant radiological lesion (Fig.1D-F) as early stage 1 (ground-glass opacities), progressive stage 2 (crazy paving pattern) or peak stage 3 (consolidation). These stages are considered as proxy for the immunological phase of COVID-19 with an early phase of active viral replication in lower airways (stage 1), progressive recruitment of pro-inflammatory cells to the lung interstitial space (stage 2), ending in diffuse alveolar damage and fibrosis (stage 3). 24.7%, 29.6% and 45.7% of patients presented in stage 1, 2 and 3, respectively with similar distribution in males and females (Table 1).

Vitamin D status in controls and COVID-19 COVID-19 patients had lower median 25(OH)D on admission (18.6 ng/mL, IQR 12.6-25.3) than a diseased control population of 2717 subjects with similar age distribution and sampled during the same season (21.5 ng/mL, IQR 13.9-20.8, P=0.0016) and a markedly higher percentage of vitamin D deficiency ((25(OH)D<20ng/mL): 58.6% versus 45.2% (P=0.0005) (Table 1). Considering the male preponderance in COVID-19 patients (59%) but underrepresentation in controls (37%), we then stratified 25(OH)D for sex (Fig.1A-C and Table 1).

Remarkably, we observed a strong sexual dimorphism. Female COVID-19 patients were not more vitamin D deficient than female controls (46.8% versus 42.8%, P=0.5646, Fig.1B). Control males showed higher vitamin D deficiency rates than control females (49.2% versus 42.8%, P<0.0001 Fig.1A). In male COVID-19 patients, vitamin D deficiency was even more profound (Fig.1C), with lower median 25(OH)D (17.6 ng/mL, IQR 12.7-24.0 versus 20.3 ng/mL, IQR 13.7-28.3, P=0.0234) and a markedly higher deficiency rate (67.0% versus 49.2%, P=0.0006) than male controls.

Correlation vitamin D status and disease stage Analysis of 25(OH)D across radiological disease stages (Fig.1G-H, Table 1) strengthened the correlation: male COVID-19 patients showed significantly lower median 25(OH)D with more advanced stage resulting in progressively increasing vitamin D deficiency rates from 55.2% in stage 1, 66.7% in stage 2 and 74.0% in stage 3 (P=0.0010). No such stage-dependent variations were seen in female COVID-19 patients.

Discussion

This retrospective observational study is to our knowledge the first to show a correlation between vitamin D deficiency, the risk to be hospitalized for severe COVID-19 pneumonia and its radiological disease stage. It reveals a remarkable sexual dimorphism, with statistical correlations in male but not female COVID-19 patients. Prospective and interventional trials are needed to define if vitamin D deficiency is cause or consequence of severe COVID-19.

Sustained inflammation could induce consumption of circulating 25(OH)D, attributed to extrarenal CYP27B1 activity and VDR expressed by the expanded repertoire of immune cells. In a USA population survey, subjects with CRP>5 mg/L showed 1.6 ng/L lower median 25(OH)D [11]. However, the stable 25(OH)D levels in female COVID-19 patients across stages argue against such effect.

The pathophysiology of severe SARS-CoV-2 infection shows similarities to that of SARS-CoV [12]: in individuals with delayed type I/III interferon response and delayed viral clearance, progressive recruitment of neutrophils and pro-inflammatory Th1/M1-polarized immune cells contribute to endothelial and alveolar cell death and in some patients trigger a cytokine storm [1] that enhances diffuse alveolar damage. Severe COVID-19 can thus be conceptualized as an unbalance between pro-inflammatory type I immune response required for viral clearance and tolerogenic type II response

required for repair [1,2,12]. Vitamin D modulates the immunological response to respiratory viruses at various phases: early on, it limits viral entry and replication by boosting cathelicidins/defensins expression in respiratory epithelia [5], later on it exerts a tolerogenic effect by directly mediating IL-4/IL-13-dependent polarization towards M2-macrophages and Th2 CD4 T cells [6]. The mammalian immune system shows conserved estrogen/androgen-dependent sexual dimorphism [13]. SARS-CoV-infected female mice show lower viral replication, lower recruitment of inflammatory monocytes and neutrophils to the lungs and less alveolar and endothelial cell death [14]. If vitamin D deficiency favors a pro-inflammatory balance, the higher rates of vitamin D deficiency in male humans might thus act in concert with estrogen/androgen-dependent immune differences and contribute to higher incidence and severity in male COVID-19 patients.

Meta-analysis confirmed that vitamin D supplementation reduces the incidence and severity of acute respiratory infections [15].

Though larger epidemiological surveys are needed, variations in vitamin D deficiencies across geographies, skin pigment (African/Latino), body mass (obese), socioeconomic status (poor) and lifestyle (institutionalized people) appear to coincide with the incidence and/or death rates of SARS-CoV-2 infections, suggesting a possible convergence of two pandemics and a global call for vitamin D supplementation as inexpensive, safe and readily available mitigation.

Conflicts of interest

The authors declare no conflict of interest

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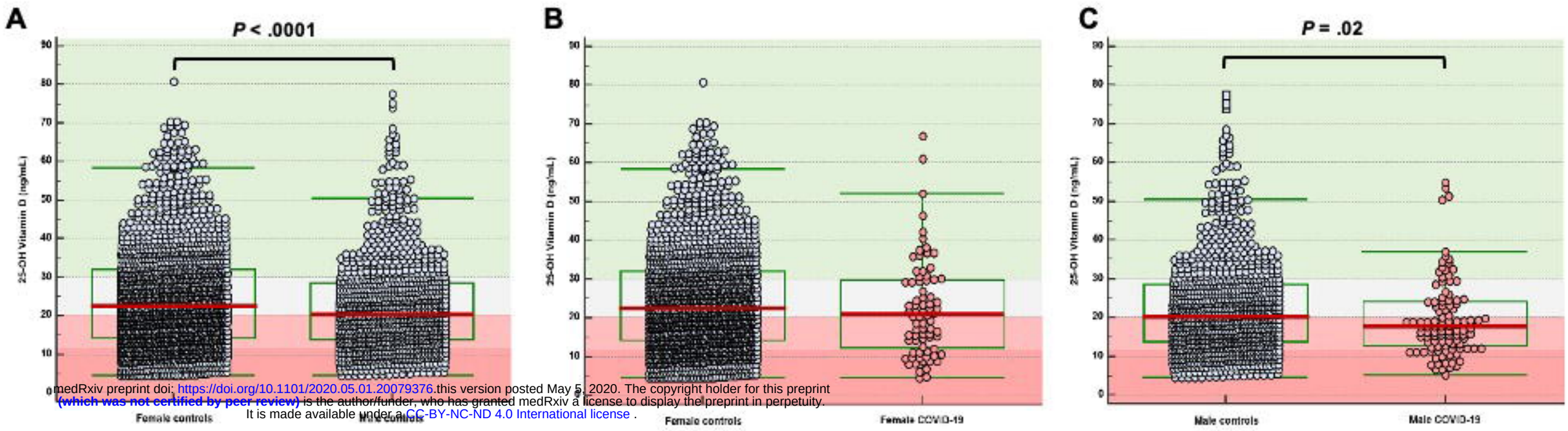
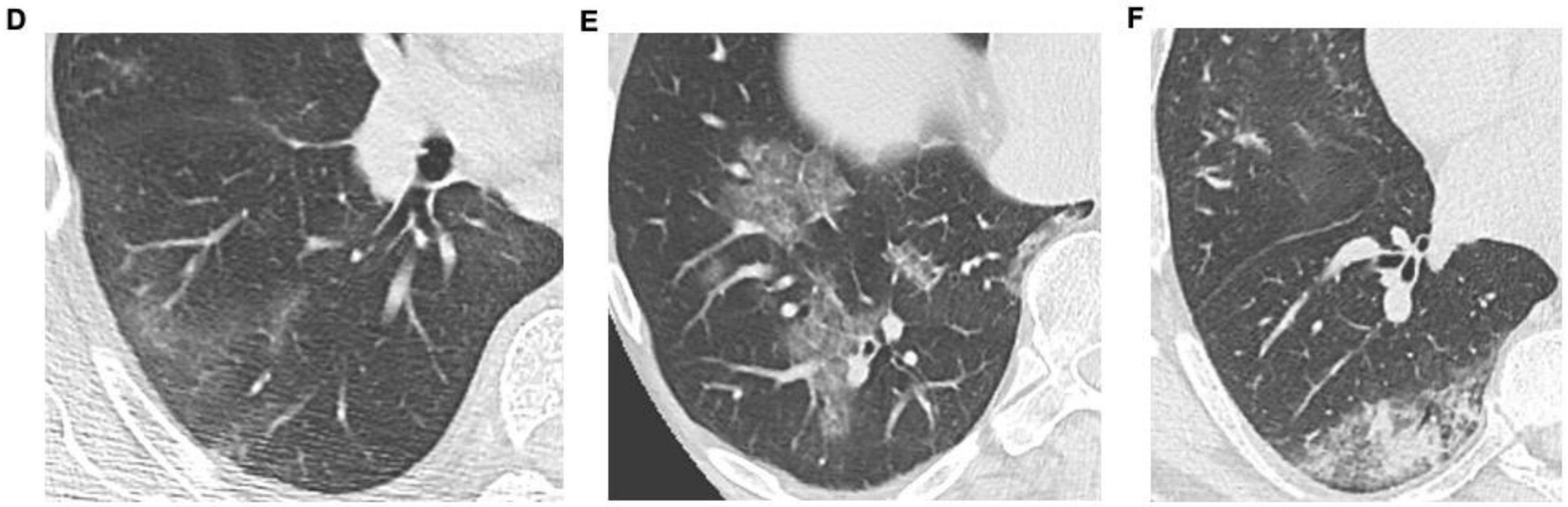
Figure legends

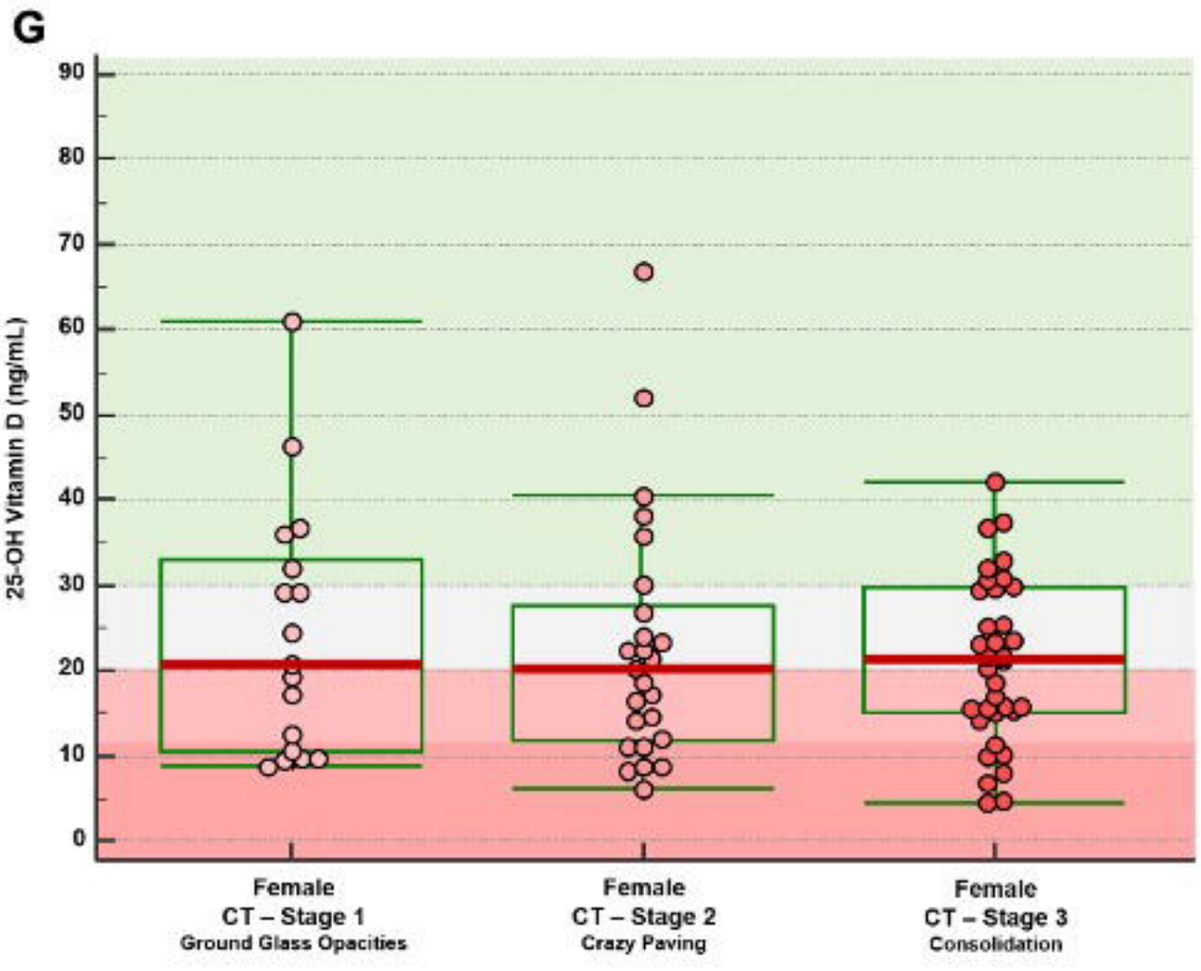
Fig.1: 25(OH)D levels in male and female COVID-19 patients and controls and stratified by radiological disease stage Panels A-C: Box-and-Whisker plots showing median (red line) serum 25(OH) levels and interquartile ranges (green box) in (A) season- and age-matched female (n=1718) versus male (n=999) diseased controls; (B) female COVID-19 patients on admission (n=77) versus female controls; (C) male COVID-19 patients on admission (n=109) versus male controls. Panels D-F: representative images of radiological stages of COVID-19 lung disease with predominantly (D) ground-glass opacities in early stage; (E) crazy paving patterns in progressive stage 2; and (F) consolidation in peak stage 3. Panel G-H: box-and-whisker plots of 25(OH)D in (G) female COVID-19 patients and (H) male COVID-19 patients grouped according to radiological stage. Background color in box plots indicates normal vitamin D status (green, 25(OH)D > 30 ng/mL), vitamin D insufficiency (pale red, 25(OH)D < 20 ng/mL), vitamin D deficiency (darker red, 25(OH)D <1 2 ng/mL) and a gray zone (20 ng/mL ≤ 25(OH)D ≤ 30 ng/mL). P values indicate statistical differences between groups calculated by Mann-Whitney test.

Table legends

Table 1. Demographic characteristics of controls and COVID-19 patients and 25(OH) levels stratified by sex and radiological stage. † Indicates differences with diseased controls for which P values less than .05 were considered statistically significant. ‡ Indicates differences with CT Stage 1 COVID-19 patients for which P values less than .05 were considered statistically significant. Data (not normally distributed) are expressed as medians (25th–75th percentiles), and the Mann-Whitney test was used to test statistical difference between groups. Proportions for categorical variables were compared using chi-squared test. All statistical analysis were performed using MedCalc and considered significant if the P value was less than .05. Exact P values listed in Supplementary information.







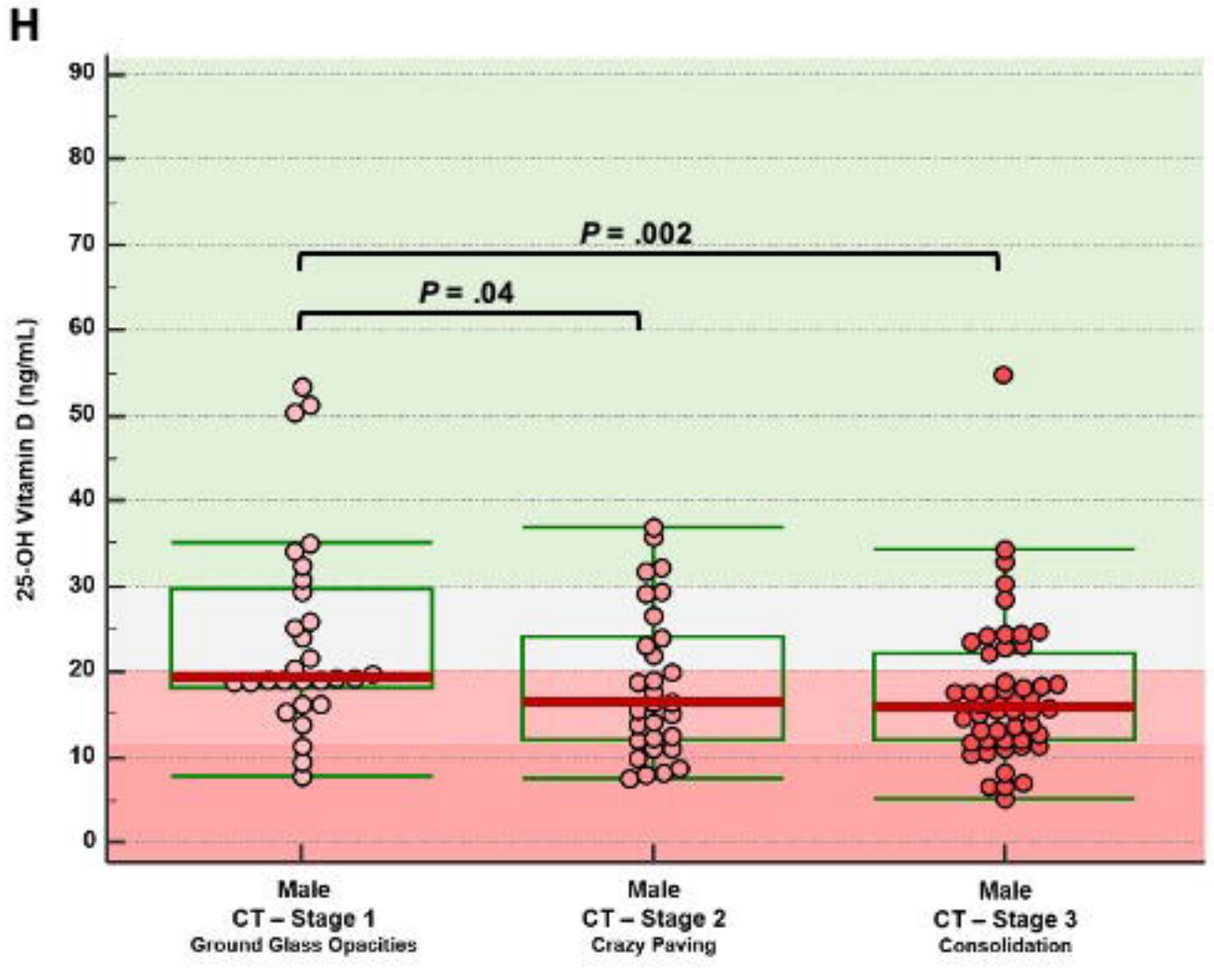


Table 1. Demographic characteristics of diseased controls and COVID-19 patients and 25(OH) levels stratified by sex and radiological COVID-19 disease stage

Patient group	Characteristic	Diseased Controls	COVID-19 (all)	COVID-19 (CT Stage 1)	COVID-19 (CT Stage 2)	COVID-19 (CT Stage 3)
All patients	n	2717	186	46	55	85
	Age, median (IQR), y	68 (49-82)	69 (52-80)	74 (53-82)	71 (60-78)	63 (50-80)
	Sex					
	Female, n (%)	1718 (63,2)	77 (41,4) †	17 (37,0) †	25 (45,5) †	35 (41,2) †
	Male, n (%)	999 (36,8)	109 (58,6) †	29 (63,0) †	30 (54,5) †	50 (58,8) †
	25-OH-Vitamin D					
	Median (IQR), ng/mL	21,5 (13,9-20,8)	18,6 (12,6-25,3) †	19,7 (16,2-30,8)	17,6 (12,0-26,0) †	16,9 (12,6-23,8) † ‡
	≥ 20 ng/mL, n (%)	1490 (54,8)	77 (41,4) †	22 (47,8)	23 (41,8)	32 (37,6) †
	< 20 ng/mL, n (%)	1227 (45,2)	109 (58,6) †	24 (52,2)	32 (58,2)	53 (62,4) †
Female patients	n	1718	77	17	25	35
	Age, median (IQR), y	68 (46-83)	71 (65-74)	68 (46-83)	72 (64-76)	66 (49-82)
	25-OH-Vitamin D					
	Median (IQR), ng/mL	22,4 (14,2-32,0)	20,7 (12,4-29,8)	20,7 (10,4-33,0)	20,3 (11,7-27,7)	21,2 (15,1-29,6)
	≥ 20 ng/mL, n (%)	983 (57,2)	41 (53,2)	9 (52,9)	13 (52,0)	19 (54,3)
	< 20 ng/mL, n (%)	735 (42,8)	36 (46,8)	8 (47,1)	12 (48,0)	16 (45,7)
Male patients	n	999	109	29	30	50
	Age, median (IQR), y	69 (53-81)	68 (53-79)	74 (58-81)	71 (59-78)	59 (52-77)
	25-OH-Vitamin D					
	Median (IQR), ng/mL	20,3 (13,7-28,4)	17,6 (12,7-24,0) †	19,4 (18,2-29,8)	16,5 (12,1-24,0) ‡	16,0 (12,0-22,1) † ‡
	≥ 20 ng/mL, n (%)	507 (50,8)	36 (33,0) †	13 (44,8)	10 (33,3)	13 (26,0) †
	< 20 ng/mL, n (%)	492 (49,2)	73 (67,0) †	16 (55,2)	20 (66,7)	37 (74,0) †

† Indicates differences with diseased controls for which P values less than .05 were considered statistically significant.
‡ Indicates differences with CT Stage 1 COVID-19 patients for which P values less than .05 were considered statistically significant.