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ORIGINAL ARTICLE

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Lower plasma calcium associated with COVID-19, but not with disease severity: a two-centre retrospective cohort study

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ABSTRACT

Background: Previous studies indicate hypocalcaemia as a potential diagnostic and prognostic marker of corona-virus disease 2019 (COVID-19). Our aim was to investigate these relations in more detail in a large test cohort and an independent validation cohort.

Methods: We retrospectively included 2792 COVID-19 suspected patients that presented to the emergency department (ED) of two hospitals. Plasma calcium and ionized plasma calcium levels were compared between COVID-19 positive and negative patients, and between severe and non-severe COVID-19 patients using univariate and multivariate analyses in the first hospital ($N = 1363$). Severe COVID-19 was defined as intensive care unit (ICU) admission or death within 28 d after admission. The results were validated by repeating the same analyses in the second hospital ($N = 1429$).

Results: A total of 693 (24.8%) of the enrolled patients were COVID-19 positive, of whom 238 (34.3%) had severe COVID-19. In both hospitals, COVID-19 positive patients had lower plasma calcium levels than COVID-19 negative patients, regardless of correction for albumin, in univariate and multivariate analysis (Δ0.06–0.13 mmol/L, $p < .001$). Ionized plasma calcium concentrations, with and without correction for pH, were also lower in COVID-19 positive patients in multivariate analyses (Δ0.02–0.05 mmol/L, $N = 567$, $p < .001$). However, we did not find a significant association between COVID-19 disease severity and plasma calcium in multivariate analyses.

Conclusions: Plasma calcium concentrations were lower in COVID-19 positive than COVID-19 negative patients but we found no association with disease severity in multivariate analyses. Further understanding of plasma calcium perturbation may facilitate the development of new preventive and therapeutic modalities for the current pandemic.

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Introduction

The COVID-19 pandemic has gripped the world. A total of 4.6 million deaths have been confirmed so far and vast numbers of new infections are reported daily [1]. It is an ongoing challenge to identify infection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, the causative virus of COVID-19) early, and to predict disease severity.

One proposed target marker for early diagnosis and prognosis is plasma calcium. Lower plasma calcium has emerged as a potential diagnostic marker of COVID-19 [2–4], while a number of studies have also linked decreased plasma calcium levels to disease severity in COVID-19 patients [5–14]. Unfortunately, studies reporting on COVID-19 and plasma calcium concentrations have limited generalizability due to several factors. First, they are subject to high study heterogeneity. Plasma calcium can be measured as total plasma calcium or biologically active ionized plasma calcium, where the former can be corrected for albumin and the latter for pH [15,16]. Most studies reported only one or two measurements of calcium, complicating the generalization of conclusions in the context of unknown mechanisms of calcium homeostasis in SARS-CoV-2 infection [2–4,6–8,11,13,17,18]. The use of study specific cut-off points to define hypocalcaemia further increases data heterogeneity and hinders clinical implementation, as well as indicating possible confirmation bias in the publication of studies of calcium-COVID-19 relations [5]. Second, most studies had a limited sample size: the largest retrospective studies were performed by Cappellini et al., including 585 patients of whom 420 were COVID-19 positive and by Di Filippo et al. including 531 COVID-19 positive patients [3,6]. Third, most studies linking COVID-19 severity to lower plasma calcium were performed in a Chinese demographic context, making generaliztion to the European context limited [7,8,11,13,14,18].

Understanding the interaction of plasma calcium homeostasis in COVID-19 may contribute to early diagnosis, identification of patients at risk for severe disease course, and the development of new preventive and therapeutic modalities. Our aim was to investigate plasma calcium levels of COVID-19 patients in more detail in a large retrospective cohort study in the Netherlands and to validate these findings in an independent cohort of patients.

Methods

Study design and setting

In this cross-sectional, retrospective cohort study we analysed 2792 suspected COVID-19 patients presenting to the emergency department (ED) of two large non-academic teaching hospitals. We first analysed 1363 patients in a test cohort from Isala hospital (Zwolle, the Netherlands) and then validated the results' reproducibility in 1429 patients in a validation cohort from the Jeroen Bosch hospital (JBZ, 's Hertogenbosch, the Netherlands).

The Medical Ethical Review Committee of Isala Zwolle declared the study not to be subject to the Medical Research Involving Human Subjects Act and waived informed consent (NWMO protocol number: 200711). The local Institutional Review Boards of both hospitals approved the study protocol.

We compared plasma calcium levels and prevalence of hypocalcaemia of COVID-19 positive patients with COVID-19 negative patients, and of severe with non-severe COVID-19 patients. Severe COVID-19 was defined as intensive care unit (ICU) admission or all-cause mortality within 28 d after hospital admission. Hypocalcaemia was defined as plasma calcium $<$2.12 mmol/L or ionized plasma calcium $<$1.17 mmol/L regardless of correction for albumin and pH, respectively [19].

Study population

All adult patients ($\geq$18 years) suspected of COVID-19 that were hospitalized after referral to the ED between 28 February 2020 and 31 January 2021 were eligible for inclusion. We regarded all patients from whom a naso-oropharyngeal swab for SARS-CoV-2 was taken as COVID-19 suspected. COVID-19 was diagnosed with a real-time reverse transcription-polymerase chain reaction (rRT-PCR) positive swab result for COVID-19.

Data collection

We retrieved patient data from the Electronic Health Record using CTCue software (CTcue B.V. version 2.2.12 and 3.5.4; Amsterdam, the Netherlands for the test and validation cohort, respectively). Collected data on clinical characteristics comprised age, sex, body mass index (BMI), hospital admission and ICU admission or death within 28 d after admission, and a modified early warning scores (MEWS) to assess the level of illness upon presentation to the ED (Supplementary Table 1) [20]. The latter was based on systolic blood pressure, heart rate, respiratory rate, and body temperature; data on level of consciousness were not available. Furthermore, we collected medication use data validated by the

hospital pharmacy upon admission from the test cohort and extracted medication that potentially influences serum calcium levels (Supplementary Table 2). We excluded patients from the test cohort if data on medication use were unavailable. Collected initial laboratory findings at ED presentation included C-reactive protein (CRP), plasma calcium, albumin, estimated glomerular filtration rate (eGFR, calculated with the CKD-EPI formula [21]), lactate dehydrogenase (LDH) and arterial blood gas analysis (pH and ionized plasma calcium). Furthermore, previous (one year to three weeks prior to ED presentation) values of calcium (25-hydroxy-)vitamin D, parathyroid hormone (PTH) and parathyroid hormone-related peptide (PTHrp) values were collected if available, to identify pre-existing calcium dysregulation. Ionized plasma calcium, MEWS and data regarding medication use were available in the test cohort but not in the validation cohort.

Biochemical analysis

In the test cohort, clinical chemistry parameters were measured using an automatic biochemical analyser (Roche Diagnostics c 501, Basel, Switzerland) and blood gas analysis was performed with the ABL90 FLEX pH-blood gas analyser (Radiometer, Copenhagen, Denmark). In the validation cohort, clinical chemistry parameters were measured using routine analysers from Siemens Healthineers (Advia XPT, Erlangen, Germany). Blood gas analysis was performed using the Rapidpoint 500 (Siemens Healthineers, Erlangen, Germany). In the validation cohort bromocresol purple (BCP) was used to measure albumin, while bromocresol green (BCG) was used in the test cohort.

We corrected the plasma calcium level for the albumin concentration using the Payne formula and standardized ionized plasma calcium to a pH of 7.4 using the Fogh-Andersen formula in the test cohort [15,16]. As there is a discrepancy between albumin measurements when using BCG and BCP, we corrected plasma calcium measurements in the validation cohort using an albumin-corrected calcium formula based on a BCP-based albumin measurement [22]. Furthermore, we corrected BCP-based albumin measurements from JBZ to BCG-based measurements (Supplementary Table 3).

Statistical analysis

All patient-specific parameters and characteristics were presented as percentages or mean ± standard deviation (SD). The Chi-square test or linear model ANOVA was used when comparing categorical and continuous variables, respectively. Next, multivariate ANCOVA analysis was used to investigate whether COVID-19 infection was a significant predictor of plasma calcium levels after correcting for all other covariates. All variables reported in this study were included as covariates except for historic laboratory values. Then, calcium differences between severe and non-severe COVID-19 patients were assessed using the same techniques. Analyses were performed with R statistical software version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). To adjust for the number of plasma calcium comparisons, Bonferroni correction was used to correct the significance threshold from 0.05 to 0.01 for individual calcium comparisons.

Results

Study population

In total, 2792 patients were included; 1363 patients in the test cohort and 1429 patients in the validation cohort. In the test cohort 26.4% (360) patients were diagnosed with COVID-19, of whom 38.6% (139) had a severe disease course. In the validation cohort, 23.3% (333) patients were COVID-19 positive, of whom 29.7% (99) had a severe disease course.

Plasma calcium and COVID-19

Average age was similar between COVID-19 positive and negative patients (69 ± 12 *vs.* 69 ± 17 years, respectively, $p = .82$; Table 1). COVID-19 positive patients were more frequently male than COVID-19 negative patients (64 *vs.* 56% male sex, $p = .008$), had a higher BMI (28.8 ± 5 *vs.* 27.3 ± 6 kg/m^2, $p < .001$) and had a higher MEWS (2.16 ± 1.63 *vs.* 1.73 ± 1.71, $p < .001$). Furthermore, COVID-19 positive patients had lower albumin (37.3 ± 4 *vs.* 39.6 ± 5 g/L, $p < .001$) and higher pH (7.48 ± 0.07 *vs.* 7.41 ± 0.11, $p < .001$). Moreover, historic calcium, PTH and vitamin D levels were similar in univariate analysis.

Uncorrected plasma calcium was lower in COVID-19 positive patients than in COVID-19 negative patients (Δ0.13 mmol/L, $p < .001$; Table 1 and Figure 1(A–D)). This difference persisted after albumin-correction of plasma calcium (Δ0.08 mmol/L, $p < .001$). Similarly, ionized plasma calcium concentrations were lower in COVID-19 positive patients before pH-correction (Δ0.05 mmol/L, $p < .001$) but this difference could not be established at the Bonferroni-corrected significance threshold after correction for pH (Δ0.02 mmol/L, $p = .03$).

Table 1. Univariate analysis of COVID-19 positive *vs.* COVID-19 negative patients.

	COVID-19 negative [$N = 1003$]	COVID-19 positive [$N = 360$]	p Value
Baseline characteristics			
Age [years]	69 (17)	69 (12)	.82
Gender [% male]	566 (56.4%)	232 (64.4%)	**.008**
BMI [kg/m^2]	27.3 (6.2) [704]	28.8 (5.2) [293]	**<.001**
MEWS	1.73 (1.71) [916]	2.16 (1.63) [349]	**<.001**
Calcium levels			
Calcium [mmol/L]	2.29 (0.16)	2.16 (0.17)	**<.001**
Calcium [mmol/L; corr.]	2.30 (0.16)	2.22 (0.16)	**<.001**
Ionized calcium [mmol/L]	1.16 (0.10) [503]	1.11 (0.06) [253]	**<.001**
Ionized calcium [mmol/L; corr.]	1.17 (0.12) [498]	1.15 (0.07) [251]	.03
Other chemistry parameters			
C-reactive protein [mg/L]	85 (99) [1001]	105 (83)	**<0.001**
eGFR [mL/min/1.73 m^2]	64 (23)	65 (21) [359]	0.34
LDH [U/L]	296 (303) [984]	381 (210) [356]	**<0.001**
Albumin [g/L]	40 (5)	37 (4)	**<0.001**
pH	7.41 (0.11) [635]	7.48 (0.07) [322]	**<.001**
Medication use[a]			
Glucocorticosteroids	95 (9.5%)	27 (7.5%)	.26
Vitamin D analogues	153 (15.3%)	50 (13.9%)	.53
Calcium-containing drugs	26 (2.6%)	5 (1.4%)	.19
Other calcium decreasing drugs	5 (0.5%)	1 (0.3%)	.59
Bisphosphonates	45 (4.5%)	19 (5.3%)	.54
Thiazide diuretics	115 (11.5%)	58 (16.1%)	**.02**
Loop diuretics	171 (17.0%)	34 (9.4%)	**<.001**
Historic chemistry measurements			
Calcium [mmol/L; 3 w–1 y prior]	2.38 (0.13) [183]	2.40 (0.12) [42]	.30
PTH [mmol/L; 3 w–1 y prior]	11.36 (9.53) [50]	10.38 (4.70) [18]	.68
Vitamin D [mmol/L; 3 w–1 y prior]	68.90 (31.11) [234]	67.93 (24.87) [73]	.81

Data from test cohort. Mean ± (standard deviation). Significant p values in bold values (<.01 for calcium measurements, otherwise < .05). Number of observations (N) in square brackets in the case of missing data.
[a]Medication in each group shown in Supplementary Table 2.

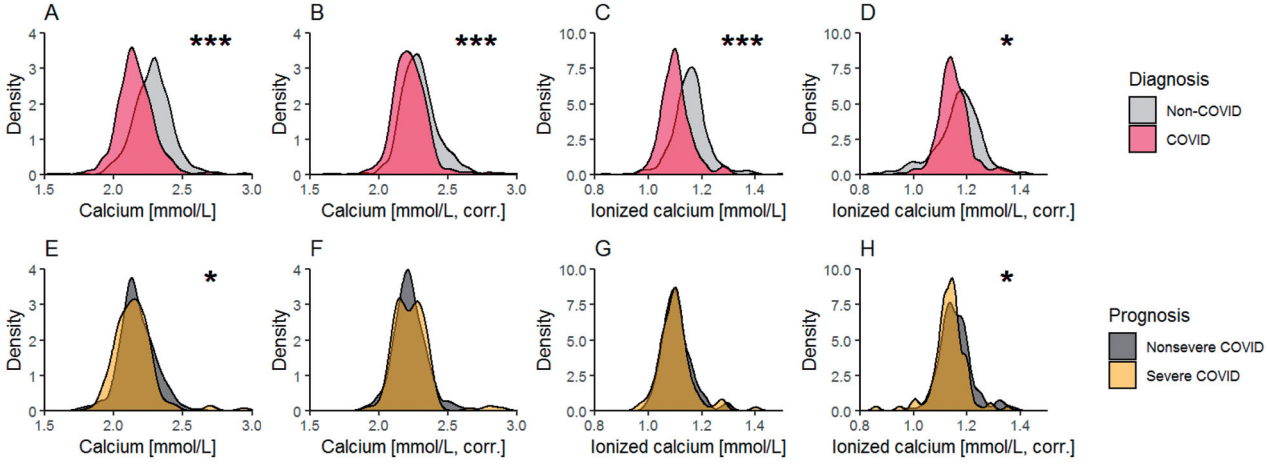

Figure 1. (A–D) distribution of plasma calcium in COVID-19 positive *vs.* COVID-19 negative patients. (E–H) distribution of plasma calcium in severe COVID-19 *vs.* non-severe COVID-19 patients. Data from test cohort. Plasma calcium on x-axis. Relative frequency of measurements on y-axis. ***p value < .001 and *p value < .05.

Additionally, hypocalcaemia was more frequent in COVID-19 positive patients than in COVID-19 negative patients. The percentages of hypocalcaemia based on plasma calcium and corrected plasma calcium of COVID-19 positive patients *vs.* COVID-19 negative patients were 37.7 *vs.* 9.9%, and 16.1 *vs.* 5.7% respectively ($p < .001$; Figure 2(A)). Hypocalcaemia prevalence based on ionized plasma calcium and corrected ionized plasma calcium was 88.9 *vs.* 56.7%, and 67.7 *vs.* 45.8% in COVID-19 positive *vs.* COVID-19 negative patients respectively ($p < .001$).

The results from the univariate analyses of calcium were consistent in multivariate analyses, where we corrected for possible spurious associations. The multivariate analyses showed a negative relationship between COVID-19 and plasma calcium levels ($N = 678$, both

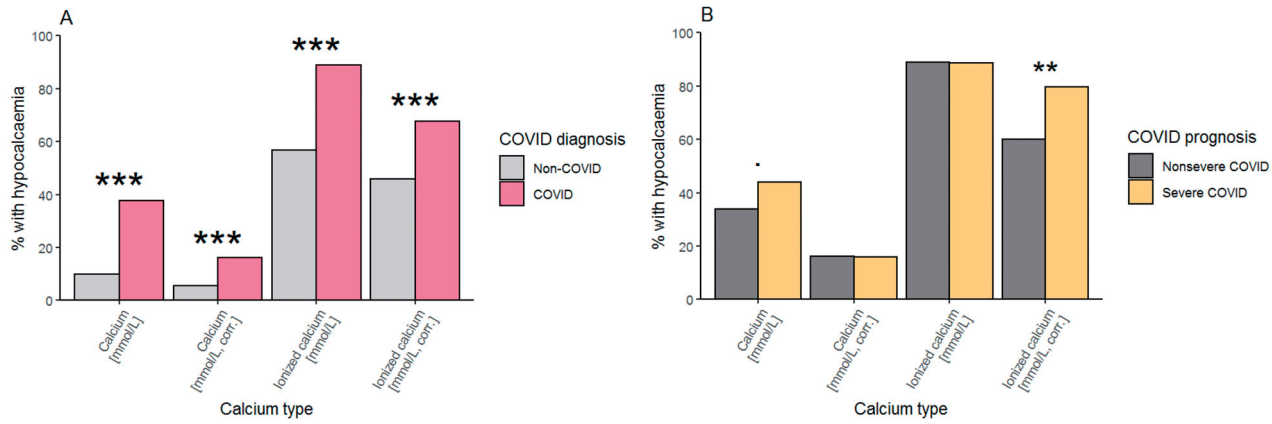

Figure 2. (A,B) Percentage of patients with hypocalcaemia. Data from test cohort. Hypocalcaemia defined as total plasma calcium <2.12 mmol/L or ionized plasma calcium < 1.17 mmol/L). ***p value < .001 and **p value < .01.

Table 2. Univariate analysis of non-severe COVID-19 *vs.* severe COVID-19.

	Non-severe COVID-19 [$N = 221$]	Severe COVID-19 [$N = 139$]	p Value
Baseline characteristics			
Age [years]	67 (13)	72 (11)	**<.001**
Gender [% male]	131 (59.3%)	101 (72.7%)	**.010**
BMI [kg/m^2]	28.6 (5.1) [184]	29.2 (5.4) [109]	.32
MEWS	1.85 (1.53) [212]	2.63 (1.68) [137]	**<.001**
ICU admission [≤28 d]	0 (0%)	69 (49.6%)	**<.001**
Mortality [≤28 d]	0 (0%)	89 (64%)	**<.001**
Calcium levels			
Calcium [mmol/L]	2.17 (0.13)	2.13 (0.21)	.04
Calcium [mmol/L; corr.]	2.22 (0.12)	2.22 (0.20)	.88
Ionized calcium [mmol/L]	1.11 (0.05) [155]	1.11 (0.08) [98]	.96
Ionized calcium [mmol/L; corr.]	1.16 (0.06) [153]	1.14 (0.07) [98]	.03
Other chemistry parameters			
C-reactive protein [mg/L]	93 (74)	124 (93)	**<.001**
eGFR [mL/min/1.73 m^2]	69 (20) [220]	59 (22)	**<.001**
LDH [U/L]	335 (147) [219]	454 (268) [137]	**<.001**
Albumin [g/L]	38 (3)	36 (4)	**<.001**
pH	7.49 (0.05) [197]	7.46 (0.09) [125]	**<.001**
Medication use[a]			
Glucocorticosteroids	12 (5.4%)	15 (10.8%)	.06
Vitamin D analogues	35 (15.8%)	15 (10.8%)	.18
Calcium-containing drugs	4 (1.8%)	1 (0.7%)	.39
Other calcium decreasing drugs	0 (0.0%)	1 (0.7%)	.21
Bisphosphonates	13 (5.9%)	6 (4.3%)	.52
Thiazide diuretics	39 (17.6%)	19 (13.7%)	.32
Loop diuretics	21 (9.5%)	13 (9.4%)	.96
Historic chemistry measurements			
Calcium [mmol/L; 3 w–1 y prior]	2.38 (0.12) [30]	2.44 (0.09) [12]	.11
PTH [mmol/L; 3 w–1 y prior]	9.93 (4.27) [12]	11.28 (5.80) [6]	.58
Vitamin D [mmol/L; 3 w–1 y prior]	66.73 (26.41) [55]	71.62 (19.63) [18]	.47

Data from test cohort. Mean ± (standard deviation). Significant p values in bold values (<.01 for calcium measurements, otherwise < .05). Number of observations (N) in square brackets in the case of missing data.
[a]Medication in each group shown in Supplementary Table 2.

$p < .001$) as well as lower ionized plasma calcium ($N = 531$, both $p < .001$), even after correction for albumin and pH (Table 3). Importantly, despite univariate differences in MEWS, pH, albumin and calcium-influencing medications, the association between lower plasma calcium levels and COVID-19 persisted after controlling for these covariates. Also, there was no significant association between any calcium measurement and MEWS ($p > .31$) or calcium-influencing medications ($p > .08$) in multivariate analyses.

Plasma calcium and disease severity

In the test cohort, 139 patients of 360 COVID-19 positive patients had a severe disease course (38.6%). 69 patients (49.6%) were admitted to the ICU and 89 patients died (64%) within 28 d after ED presentation. Patients with severe COVID-19 were older (72 ± 11 *vs.* 67 ± 13 years, $p < .001$), were more frequently male (73 *vs.* 59% male sex, $p = .010$), had similar BMI (29.2 ± 5 *vs.* 28.6 ± 5 kg/m^2, $p = .32$) and had a higher MEWS (2.63 ± 1.68 *vs.*

Table 3. Summary of performed tests and significance in multivariate analyses.

		Test cohort			Validation cohort		
Covariate	Outcome variable	*n*	Coefficient	*p* Value	*n*	Coefficient	*p* Value
COVID-positive *vs.* negative	Calcium [mmol/L]	678	−0.099	**<.001**	661	−0.039	.014
	Calcium [mmol/L; corr.]	678	−0.099	**<.001**	661	−0.039	.014
	Ionized calcium [mmol/L]	531	−0.045	**<.001**	0	–	–
	Ionized calcium [mmol/L; corr.]	531	−0.086	**<.001**	0	–	–
Severe COVID *vs.* non-severe	Calcium [mmol/L]	253	−0.003	.85	172	−0.012	.59
	Calcium [mmol/L; corr.]	253	−0.008	.85	172	−0.012	.59
	Ionized calcium [mmol/L]	197	0.008	.39	0	–	–
	Ionized calcium [mmol/L; corr.]	197	0.008	.42	0	–	–

Significant *p* values in bold values (<.01 after Bonferroni-correction). All data mentioned in Tables 1 and 2 included as covariates except for medication use in the validation cohort.

1.85 ± 1.53, $p < .001$). Furthermore, patients with severe COVID-19 had lower albumin concentrations (36.4 ± 4 *vs.* 37.8 ± 3 g/L, $p < .001$) and lower pH (7.46 ± 0.09 *vs.* 7.49 ± 0.05, $p < .001$ $p = .03$; Table 2).

In univariate analyses, there was a trend towards lower uncorrected total calcium and lower pH-corrected ionized calcium levels in severe COVID-19 patients (Δ0.03 mmol/L, $p = .04$ and Δ0.02 mmol/L, $p = .03$; Table 2 and Figure 1(E–H)). Albumin-corrected total calcium and uncorrected ionized plasma calcium levels were similar in both groups ($p = .88$ and $p = 1$, respectively). Hypocalcaemia percentages were similar between both groups, except for when based on pH-corrected ionized calcium (79.6% in severe and 60.1% in non-severe COVID-19 patients, $p < .01$; Figure 2(B)). Additionally, we assessed whether ≤28 d ICU admission or ≤28 d mortality were individually associated with lower calcium levels in our test cohort. We found no differences in plasma calcium levels between these groups (all $p > .07$).

Finally, as uncorrected plasma calcium and pH corrected ionized calcium were close to our Bonferroni-corrected significance threshold in univariate analyses, we also performed multivariate analysis of these measurements but were not able to demonstrate an association with disease severity ($N = 253$, $p = .85$ and $N = 197$, $p = .42$, respectively; Table 3).

Validation cohort

In the validation cohort of 1429 patients, 333 (23.3%) COVID-19 positive patients were included, 99 of whom had a severe disease course (42.5%). In general, plasma calcium levels were lower in the test cohort than in the validation cohort (Δ0.09 mmol/L, respectively, $p < .001$).

In the validation cohort, plasma calcium levels (uncorrected and corrected) were also lower in COVID-19 patients compared to non-COVID-19 patients (Δ0.08 and 0.06 mmol/L respectively, both $p < .001$, Supplementary Table 4). Although the association of COVID-19 and lower plasma calcium showed a tendency in multivariate analyses this was not significant when applying Bonferroni correction ($N = 661$, both $p = .014$) (Table 3).

Furthermore, no correlation between COVID-19 severity and calcium levels in the validation cohort was found. Severe and non-severe COVID-19 patients had similar uncorrected plasma calcium levels (Δ0.01 mmol/L, $p = .45$) and corrected plasma calcium levels (Δ0.01 mmol/L, $p = .33$, Supplementary Table 5). Ionized calcium was not routinely measured at the ED in the validation cohort, so analyses for ionized plasma calcium could not be performed.

Discussion

In this study, we demonstrated that plasma calcium, independent of plasma albumin concentration, was lower in COVID-19 positive than in COVID-19 negative patients in two independent hospitals. Similarly, there was an association between COVID-19 and lower uncorrected ionized plasma calcium levels and a trend towards lower corrected ionized plasma calcium levels. Multivariate analysis showed unambiguous negative association between all plasma calcium levels and COVID-19. However, we were unable to demonstrate a significant association between plasma calcium levels and disease severity in multivariate analysis based on ICU admission or mortality within 28 d after admission. To our knowledge, this is the largest study so far to assess the relation of plasma calcium and COVID-19 using multivariate analysis, and it is the first to include an independent validation cohort.

So far, three studies have compared plasma calcium levels between COVID-19 positive patients and a control group [2–4]. These studies suggest a possible relationship between SARS-CoV-2 infection and lower plasma calcium levels but have several limitations. Two retrospective studies had a limited sample size, including 40 and 50 patients, respectively [2,4]. The only large

retrospective study to date by Cappellini et al. analysed 585 COVID-19 suspected patients (72% COVID-19 positive) and found a difference of Δ0.13 and Δ0.05 mmol/L in uncorrected plasma calcium and uncorrected ionized plasma calcium, respectively, when comparing non-COVID-19 to COVID-19 patients [3]. In our study, we were able to replicate the findings of Cappellini et al. but in addition to patient age, sex, and pH as covariates, we were able to control for MEWS, biochemical parameters and calcium-interfering medication.

Two covariates of particular interest in our study are the MEWS and pH. First, our univariate analysis of the test cohort shows that COVID-19 positive patients had a higher MEWS. As hypocalcaemia is reported to be associated with more critically ill patients this suggests that the difference in calcium levels may be explained by the level of illness rather than by SARS-CoV-2 infection [23]. However, despite multivariate control for the MEWS, we found persistent association of COVID-19 with calcium levels and the MEWS was not a predictor of calcium in these models ($p > .31$).

Second, we found higher pH levels in COVID-19 positive patients than in COVID-19 negative patients (7.48 ± 0.07 *vs.* 7.41 ± 0.11, $p < .001$); this is a known phenomenon in COVID-19 patients primarily contributed to hypoxia-driven respiratory alkalosis [24]. Although errors in sample procedure may cause pH drift in blood samples [25], pH values in our study are likely to represent true blood pH of patients as all blood gas analyses in our ED were arterial, were drawn in anaerobic conditions, and were processed urgently. Because alkalosis lowers the amount of free ionized calcium in the blood due to increased albumin binding, differences in blood pH partially explain the difference in ionized calcium levels between COVID-19 positive and negative patients [25]. This effect is illustrated by the attenuation of the difference of uncorrected ionized calcium levels (Δ0.05 mmol/L, $p < .001$) after correction for pH (Δ0.02 mmol/L, $p = .03$) in univariate analysis (Table 2). However, the multivariate analyses show an association between ionized calcium and COVID-19 that persists even after adding pH to the model ($p < .001$; Table 3). A significant association between pH and ionized calcium was present in this model ($p = .04$), but did not decrease the validity of the relationship between ionized calcium and COVID-19, leading us to believe that despite an effect of pH, lower ionized calcium levels are associated with SARS-CoV-2 infection independently.

Most studies describing the relation between plasma calcium and COVID-19 focus on relating calcium to disease severity and report that lower plasma calcium is associated with more severe disease [5–8,10–14]. However, these studies too have several limitations, and we were not able to replicate their findings as we did not find a correlation between lower levels of plasma calcium and COVID-19 severity. The most likely explanation is that several of these studies performed only univariate comparisons of calcium levels or prevalence of hypocalcaemia, leaving the influence of covariates unaccounted for [8,14,18]. Two of the studies that used multivariate analyses found an association between lower plasma calcium and hospitalization [6] and hospitalization ≥14 d [13] but not with ICU admission or mortality as we assessed in our study. However, to the best of our knowledge, only one small study found an independent association between hypocalcaemia (defined as albumin-corrected plasma calcium < 2.15 mmol/L) and poor outcome (composite of ICU admission, invasive mechanical ventilation, or death) in 107 patients (OR 2.962, 95% CI [1.085–8.090], $p = .034$) [7].

Another limitation is that most studies to date use hypocalcaemia with different cut-off points as their primary endpoint instead of actual plasma calcium levels [6,8,11–13]. Although the use of cut-off values makes for easier translation to clinical practice, the lack of a uniform definition may lead to arbitrary and potentially biased data reporting.

All in all, the multitude of definitions of hypocalcaemia and of used calcium measurements (plasma calcium and ionized plasma calcium, with and without correction) in previous studies in combination with limited control for covariates limits the persuasion of current evidence claiming a relationship between COVID-19 severity and plasma calcium. Although the design of this study was not aimed at excluding such a relationship, we believe that in the light of the results of this study, such a relation should not be assumed unless new studies provide other compelling evidence.

We recognize that this study has some limitations with consequences for the generalization of our findings. Due to the retrospective design of our study, it is possible that there is a selection bias. However, by using multivariate analyses we corrected for the most important covariates limiting this bias. Furthermore, the fact that the associations in our results were similar in two independent datasets, despite inter-hospital variability of plasma calcium levels due to different biochemical equipment, increases generalizability.

Second, there was no information about the disease stage of patients upon admission. However, if plasma

calcium perturbation would be time-specific during infection, this would lead to higher interpatient variation, ultimately causing an underestimation of the impact of COVID-19 on plasma calcium in our study. Therefore, it is more likely that between-group differences can be attributed to the presence of COVID-19 than to variety in disease stage in our population.

Finally, our study provides no mechanistic insight in how plasma calcium perturbation is mediated. Although with limited observations, we found that historic calcium, PTH and vitamin D levels were similar in COVID-19 positive and COVID-19 negative patients, implying that lower plasma calcium is an acute effect associated with a COVID-19 infection and not mediated by pre-existing dysregulation that changes susceptibility to the disease.

One hypothesis for lower plasma calcium in COVID-19 is that calcium may be depleted as it is used in viral replication and host cell entry. This effect was also described in other coronaviruses with similar calcium-dependent fusion loop domains in the spike protein [26–28]. Alternatively, hypocalcaemia may be caused by calcium-binding of unsaturated fatty acids (UFA's) released in severe COVID-19 [29,30]. Furthermore, lowered plasma calcium can be attributed to changes in PTH, vitamin D and albumin or by direct calcium depletion. From a physiological standpoint, PTH insufficiency seems less likely as this causes hyperphosphataemia, whereas COVID-19 is associated with hypophosphataemia [31]. However, several studies have reported vitamin D insufficiency as a risk factor for COVID-19 and some have even linked it to hypocalcaemia in COVID-19 patients, making hypovitaminosis D one of the more likely causes of hypocalcaemia in COVID-19 [32–35].

Plasma calcium is an underappreciated diagnostic marker of COVID-19, possibly because the mean differences between COVID-19 positive and negative patients are small, making it difficult to use in a clinical context. However, the understanding of underlying mechanisms of plasma calcium perturbation during COVID-19 can guide targeted preventative and therapeutic interventions, such as vitamin D, albumin, or calcium supplementation, and the use of calcium channel blockers [36].

Conclusion

In this study, we demonstrated that COVID-19 patients have significantly lower plasma calcium and ionized plasma calcium levels than patients without COVID-19 in two independent cohorts. We were unable to demonstrate a significant relationship between reduced plasma calcium levels and COVID-19 disease severity after correction for covariates, despite a univariate trend between disease severity and uncorrected total calcium and pH-corrected ionized calcium. We believe that expanding knowledge of the mechanistic basis will lead to new avenues in the development of new therapeutic and preventive treatments for the current (and possibly future) coronavirus pandemic(s).

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Disclosure statement

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