# Serum Calcium, Magnesium, and Phosphorus Levels in Patients with COVID-19: Relationships with Poor Outcome and Mortality

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## ABSTRACT

In this retrospective study to assess the impact of serum corrected calcium (CorrCa), magnesium (Mg) and phosphorus (P) levels, all adult patients with laboratory-confirmed COVID-19 hospitalized during 2020 were included. Poor outcome was considered in patients who presented need for mechanical ventilation, intensive care unit (ICU) admission, or in-hospital mortality. We analyzed 2473 patients (956 females) aged (mean ± SD) 63.4 ± 15.9 years. During admission, 169 patients (6.8 %) required mechanical ventilation, 205 (8.3 %) were admitted to the ICU, and 270 (10.9 %) died. Composite variable of poor outcome, defined as need for mechanical ventilation, ICU admission or death, was present in 434 (17.5 %) patients. In univariate analysis, the need for mechanical ventilation was positively related to Mg levels (OR 8.37, 95 % CI 3.62–19.33; p < 0.001); ICU admission was related to CorrCa (OR 0.49, 95 % CI 0.25–0.99; p = 0.049) and Mg levels (OR 5.81, 95 % CI 2.74–12.35; p < 0.001); and in-hospital mortality was related to CorrCa (OR 1.73, 95 % CI 1.14–2.64; p = 0.011). The composite variable of poor outcome was only related to Mg (OR 2.68, 95 % CI 1.54–4.68; p = 0.001). However, in multivariate analysis only CorrCa was significantly related to the need for mechanical ventilation (OR 0.19, 95 % CI 0.05–0.72; p = 0.014) and ICU admission (OR 0.25; 95 % CI 0.09–0.66; p = 0.005), but not with in-hospital mortality or the composite variable. In conclusion, CorrCa can be used as a simple and reliable marker of poor outcome in patients with COVID-19, although not to predict the risk of in-hospital mortality.

## Introduction

During the last months, the evolution of the pandemic caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has been accompanied by a growing interest in defining the endocrine and metabolic disorders associated with coronavirus disease 2019 (COVID-19) [1]. Simultaneously, attempts have been made to search for risk factors for severe illness or death that allow clini-

cians to identify groups of patients that are most likely to have poor outcomes [2].

It is known that calcium (Ca) plays a role in viral infection and participates in the replication mechanisms of SARS-CoV-2 and other viruses such as Middle East respiratory syndrome coronavirus (MERS-CoV) and Ebola [3–5]. Some clinical studies have shown that hypocalcemia is common during the admission of patients admitted for viral infections [6]. The first case of COVID-19 associated with severe hypocalcemia was reported by Bossoni et al. [7] during the first months of the pandemic. Subsequently, di Filippo et al. [8] found hypocalcemia in a strikingly high proportion (78 %) of patients with SARS-CoV-2 infection. In this study, hypocalcemia was a risk factor for hospitalization of the patients, although it was not possible to demonstrate whether hypocalcemia was an independent risk factor for admission to the intensive care unit (ICU) or mortality. Other studies have shown that hypocalcemia in patients with COVID-19 has been associated with higher values of white blood cell count, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6) and D-dimer, and reduced levels of lymphocytes and albumin [9]. Low Ca levels have also been associated with a higher incidence of organ damage, septic shock, worse prognosis, and higher short-term mortality [9, 10].

Although several of the studies published so far [8–13] showed a relationship between hypocalcemia and COVID-19, many knowledge gaps remain in the relationship that might exist between SARS-CoV-2 infection and Ca metabolism. For instance, we do not know with precision what are the poor outcome results independently conditioned by hypocalcemia or if there is any influence of serum magnesium (Mg) and phosphorus (P) concentrations on COVID-19 prognosis. Therefore, our aim in this study has been to investigate whether there is a relationship between alterations in serum concentrations of Ca metabolism parameters [Ca, corrected Ca (CorrCa)), Mg, and P] with the severity of the disease, as well as in-hospital mortality.

## Patients and Methods

### Patients

We conducted a retrospective study to assess Ca metabolism in all patients with diagnosis of severe COVID-19 admitted to the Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) during 2020. All adult patients included in this study presented the diagnosis of COVID-19 based on the clinical and radiological criteria established by the WHO [14]. Confirmation was made by analysis of nasopharyngeal swab samples for detection of SARS-CoV-2 viral nucleic acid by reverse transcription-polymerase chain reaction (RT-PCR) assay [15]. The inclusion criteria were: age ≥ 18 years, hospital admission from January 1st, 2020 and hospital discharge before January 1st, 2021, COVID-19 diagnosis confirmed by RT-PCR and access to information on drug prescription from 6 months before the date of admission. To avoid including patients with disorders that are usually associated with altered Ca, P, and Mg levels, we excluded patients with the following conditions: hyperparathyroidism, hypoparathyroidism, osteoporosis, severe chronic kidney disease (CKD), defined as estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 $m^2$, and therapy with calcium or vitamin D during the 6 months prior to admission.

This study was conducted in accordance with the Declaration of Helsinki; it was approved by the ethics committee of HUPHM (Institutional Review Board number 2020/231). The requirement for written informed consent was waived given the retrospective nature of this study.

### Study design

The strategy for capturing patients participating in this study was a systematic capture from structured electronic medical records facilitated by the Department of Admission and Clinical Documentation of the HUPHM. To create this database the APR-GRD Patient Classification System (All Refined Patients – Diagnosis Related Groups, IAmetrics program, version 32.0) was used [16]. This information subsystem included the coding of the Hospitalization Discharge Reports that was carried out in the Coding Unit of the HUPHM, using the International Classification of Diseases ICD-10-ES, ed. 2020, and the standards established for the assignment of codes by the Spanish Ministry of Health [17].

The database was interrogated to establish the total study population with the following filters: Patients with an admission date between 01/01/2020 and 31/12/2020, with a discharge date equal to or less than 31/12/2020; patients ≥ 18 years old; admission to any department of the hospital; patients who in the Minimum Basic Data Set had the codes that identify the SARS-CoV-2 coronavirus infection in the ICD-10-ES. Once the database with the total population under study was established, the following filters were applied to it in all the fields that collect diagnostic and procedural codes to identify the presence of comorbidities and the use of mechanical ventilation: diabetes, E08, E09, E10, E11; obesity, E66, O99.21; cardiac ischemia, codes included in Sections I20-I25; hypertension, I10, I11, I12, I13, I15, I27.0; hypoparathyroidism, E20, E89.2; hyperparathyroidism, E21, N25.81; osteoporosis, M80, M81; and mechanical ventilation, 5A1935Z, 5A1945Z, 5A1955Z.

### Studied variables

We registered the following demographic and clinical variables in all patients: gender, age, duration of hospitalization, and comorbidities [diabetes, obesity, hypertension and coronary heart disease (CHD)]. The analytical data collected in this study were those obtained during the first 24 hours after admission to the hospital, either in the emergency department or on the hospital ward. We focus on parameters related to calcium metabolism, that is, serum concentrations of Ca, CorrCa, Mg and P. We also selected laboratory values previously described as relevant for the disease, that is, renal function markers [serum creatinine, estimated glomerular filtration rate (eGFR)], blood count parameters (leukocyte and lymphocyte count), inflammatory markers (albumin, CRP, procalcitonin, and IL-6), hemostasis parameters (D-dimer), liver and cardiac function markers [N-terminal pro-B-type natriuretic peptide (NT-proBNP), lactate dehydrogenase (LDH), troponin I], and gasometric parameters [pH and arterial oxygen saturation ($SpO_2$)].

Since this is a retrospective study, the retrieved analytical information was that was requested by the responsible physicians according to the clinical needs of the patients and was available on the electronic clinical records. Therefore, some laboratory param-

eters were not available in all patients. Assessed outcomes included the need for mechanical ventilation, ICU admission and death. We created a composite variable to assess the poor outcome of the patients. This composite variable was formed by the combination of the previous three, that is, need for mechanical ventilation, ICU admission or in-hospital death.

### Laboratory procedures

Serum concentrations of Ca, Mg, P, and the rest of analytical parameters used in this study were carried out by the Department of Clinical Biochemistry of the HUPHM through standard procedures (see Supplementary Material for details). The reference intervals of our laboratory were considered as normal values (▶ **Table 1**). When available, we calculated serum Ca corrected by albumin according to the following formula: CorrCa (mg/dl) = Ca (mg/dl) + 0.8 [4-albumin (g/dl)]. eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [18].

### Statistical analysis

For quantitative variables, results are expressed as mean ± SD for normally distributed data and as median (interquartile range) for nonparametric data. Adjustment to normal distribution was tested by the Kolmogorov test. Categorical variables are described as ratios or percentages (%). For comparisons of means between two groups of subjects, the Student's *t*-test was used for normally distributed data and the Mann-Whitney U-test was employed for nonparametric data. For ratio comparisons, the chi-squared test or Fisher's exact test was used. Several models of logistic regression analysis were used to assess the dependence of the poor outcome variables (mechanical ventilation, ICU admission, mortality and composite variable) as a function of diverse quantitative and qualitative variables. The univariate analysis included demographic variables and comorbid conditions (n = 2473), as well as all the main target parameters of our study (CorrCa, n = 1466; Mg, n = 859; and P, n = 1274). Since not all patients had all the laboratory results considered in the study, for the univariate analysis, we selected the most representative parameters known as risk factors for poor outcome in COVID-19 patients and excluded those with a sample size of < 1400. In the multivariate analysis, all the variables with a value of $p < 0.10$ in the univariate model were included. Given the existence of missing values in this cohort of patients, logistic regression analysis was performed with the available sample size for each variable studied, that is, without imputation of mean values in any variable. When one of the calcium metabolism variables was significant in the prediction of any of the events studied in the multivariate analysis, we used the receiver operating characteristic (ROC) curve analysis to define the power of the event prediction. All used tests were two-sided, and differences were considered significant when $p < 0.05$.

## Results

### Patients' characteristics and laboratory values

During the study period, 2736 patients were admitted to HUPHM because of COVID-19. After excluding 263 patients with hyperparathyroidism (n = 45), hypoparathyroidism (n = 5), osteoporosis (n = 95), CKD (n = 101) or therapy with calcium or vitamin D (n = 57), we studied a sample of 2473 patients whose main clinical and biochemical characteristics are summarized in ▶ **Table 1**. Mean age (± SD) of studied patients was 63.4 ± 15.9 years and there were 956 women (38.7 %) and 1517 men (61.3 %). Main comorbidities were hypertension (42.4 %), diabetes (18.6 %), obesity (9.9 %), and CHD (8.4 %). At admission, severity of the disease was mild or moderate in 48.2 % and severe or extreme in 51.7 % of patients. Median length of stay was 7.0 (4.0–13.0) days.

Biochemical parameters of Ca metabolism, as well as the most relevant laboratory values related to the COVID-19 disease are detailed in ▶ **Table 1**. On initial evaluation, mean Ca, CorrCa, Mg and P levels were, respectively, 8.55 ± 0.51 (n = 1820), 8.80 ± 0.44 (n = 1466), 2.07 ± 0.34 (n = 859) and 3.27 ± 0.72 (n = 1274) mg/dl, in patients with available data. Hypocalcemia, defined as levels of Ca or CorrCa < 8.7 mg/dl, was found in 57.5 and 39.4 % of patients, respectively. Hypermagnesemia, defined as Mg levels > 2.67 mg/dl, was found in 1.9 % of subjects and, lastly, hypophosphatemia, defined as P levels < 2.5 mg/dl, was found in 12.1 % of them.

### Outcome of patients

During admission, 169 patients (6.8 %) required mechanical ventilation and 205 (8.3 %) were admitted to the ICU. Lastly, 270 (10.9 %) patients died in the hospital (▶ **Table 1**). Overall, 434 (17.5 %) patients had a poor outcome. As shown in **Table S1** (**Supplementary Material**), the poor outcome variables and the composite variable were significantly related to the age, male gender, length of stay, severity of the disease at admission, and the majority of studied comorbid conditions.

On the other hand, **Tables S2** to **S5** (**Supplementary Material**) show the COVID-19-related laboratory parameters in patients classified according to the need for mechanical ventilation, ICU admission, in-hospital mortality, and the composite variable. Both mortality and composite variable were significantly related to all known poor prognostic factors of COVID-19, that is, decreased kidney function, leukocytosis, lymphopenia, hypoalbuminemia, elevation of inflammatory markers (CRP, procalcitonin and IL-6), altered coagulation (elevated D-dimer), altered muscle and cardiac function markers (NT-proBNP, LDH, troponin), and decreased pH and arterial oxygen saturation.

### Calcium metabolism and poor outcome

Patients who required mechanical ventilation or ICU admission had significantly lower serum Ca values and significantly higher serum Mg values than those who did not have these requirements. Patients who died during admission exhibited lower serum Ca values, but higher CorrCa values than patients who did not die. Lastly, the group of subjects with the composite variable showed lower serum Ca (but not CorrCa) levels and higher Mg levels than patients without this variable (▶ **Table 2**).

The relationship between derangements in Ca metabolism and the poor outcome are shown in **Table S6** (**Supplementary Material**). Hypocalcemia, defined by serum Ca values, was significantly related to all poor prognostic variables ($p < 0.001$ in all cases). However, when hypocalcemia was defined by CorrCa, the significant relationship only persisted in the case of mechanical ventilation ($p = 0.026$). On the other hand, hypermagnesemia was significant-

▶ **Table 1** Clinical and biochemical characteristics of patients with COVID–19 included in the study.

| | | All admitted patients | | Included patients | | |
|---|---|---|---|---|---|---|
| **Variable** | **Units or categories** | **No.** | **Value** | **No.** | **value** | **Interval of reference** |
| **Demographic** | | | | | | |
| Sex | Female | 2736 | 1116 (40,8 %) | 2473 | 956 (38.7) | |
| Age | Years | 2736 | 64.8 ± 16.3 | 2473 | 63.4 ± 15.9 | |
| **Comorbidities** | | | | | | |
| Diabetes | | 2736 | 534 (19.5) | 2473 | 460 (18.6) | |
| Obesity | | 2736 | 274 (10.0) | 2473 | 246 (9.9) | |
| Hypertension | | 2736 | 1231 (45.0) | 2473 | 1048 (42.4) | |
| CHD | | 2736 | 244 (8.9) | 2473 | 207 (8.4) | |
| CKD | | 2736 | 101 (3.7) | | | |
| Hypoparathyroidism | | 2736 | 5(0.2) | | | |
| Hyperparathyroidism | | 2736 | 45 (1.6) | | | |
| Osteoporosis | | 2736 | 95 (3.5) | | | |
| Ca or vitamin D therapy | | 2736 | 57 (2.1) | | | |
| Severity | Mild | 2736 | 536 (19.6) | | 515 (20.8) | |
| | Moderate | | 726 (26.5) | | 678 (27.4) | |
| | Severe | | 972 (35.5) | | 847 (34.2) | |
| | Extreme | | 502 (18.3) | | 433 (17.5) | |
| **Laboratory results** | | | | | | |
| Ca | mg/dl | 2019 | 8.54 ± 0.53 | 1820 | 8.55 ± 0.51 | 8.7–10.3 |
| CorrCa | mg/dl | 1638 | 8.80 ± 0.46 | 1466 | 8.80 ± 0.44 | 8.7–10.3 |
| Mg | mg/dl | 966 | 2.07 ± 0.34 | 859 | 2.07 ± 0.34 | 1.46–2.67 |
| P | mg/dl | 1439 | 3.32 ± 0.84 | 1274 | 3.27 ± 0.72 | 2.5–4.5 |
| Creatinine mg/dl | | 2222 | 0.77 (0.58–0.99) | 2005 | 0.76 (0.58–0.96) | 0.6–1.2 |
| eGFR | ml/min/1.73 $m^3$ | 1702 | 89 (71 –>90) | 1505 | 91 (77 –>90) | >90 |
| Leucotytes | × $10^3$/µl | 2416 | 7.14 (5.23–9.64) | 2177 | 7.12 (5.25–9.64) | 4.0–11.5 |
| Lymphocytes | × $10^3$/µl | 2417 | 1.01 (0.72–1.41) | 2178 | 1.04 (0.73–1.43) | 1.2–4.0 |
| Albumin | g/dl | 1924 | 3.69 ± 0.41 | 1719 | 3.71 ± 0.40 | 3.5–5.0 |
| CRP | mg/l | 2296 | 67.8 (27.5–129.2) | 2068 | 67.1 (27.1–127.4) | 0.1–10.0 |
| Procalcitonin | ng/ml | 428 | 0.08 (0.04–0.22) | 387 | 0.08 (0.03–0.19) | 0.00–0.10 |
| IL-6 | pg/ml | 941 | 99.0 (29.0–345.5) | 933 | 98 (28–346) | 0.0–4.4 |
| D-dimer | µg/ml | 2589 | 0.90 (0.54–1.72) | 2344 | 0.86 (0.53–1.63) | 0–0.5 |
| NT-proBNP | pg/ml | 1170 | 370 (100–1583) | 1005 | 281 (87–1077) | 10–125 |
| LDH | U/l | 2178 | 257 (176–339) | 1959 | 257 (177–338) | 120–246 |
| Troponin | µg/l | 1419 | 0.017 (0.017–0.020) | 1294 | 1294 (0.017–0.020) | 0.00–0.06 |
| pH | | 1644 | 7.45 ± 0.05 | 1490 | 7.75 ± 0.05 | 7.35–7.45 |
| $SpO_2$ | % | 1645 | 93.3 (60.3–95.6) | 1491 | 93.3 (90.5–95.5) | 95.0–98.0 |
| **Hospital admission** | | | | | | |
| Length of stay | Days | 2736 | 7.0 (4.0–13.0) | 2473 | 7.0 (4.0–13.0) | |
| Mechanical ventilation | | 2736 | 178 (6.5) | 2473 | 169 (6.8) | |
| ICU admission | | 2736 | 220 (8.0) | 2473 | 205 (8.3) | |
| Days in ICU | Days | 220 | 15.5 (6.0–29.8) | 205 | 16.0 (6.0–30.0) | |
| Discharge | Home | 2736 | 2325 (85.1) | 2473 | 2141 (86.5) | |
| | Transfer to another hospital | | 66 (2.4) | | 62 (2.5) | |
| | Exitus | | 343 (12.5) | | 270 (10.9) | |

No.: Total number of patients with available data; CHD: Coronary heart disease; CKD: Chronic kidney disease; Ca: Calcium; CorrCa: Corrected calcium; Mg: Magnesium; P: Phosphorus; eGFR: Estimated glomerular filtration rate calculated with the CKD-EPI formula.; CRP: C-reactive protein; IL-6: interleukin-6; NT-proBNP: N-terminal pro-B-type natriuretic peptide; LDH: Lactate dehydrogenase; $SpO_2$: Arterial oxygen saturation; ICU: Intensive care unit.

▶ **Table 2** Laboratory parameters of calcium metabolism in patients classified according to the need for mechanical ventilation, ICU admission, in-hospital mortality, and the composite variable of poor prognosis.

| | n | Value | n | Value | p |
|---|---|---|---|---|---|
| **Need for mechanical ventilation** | | | | | |
| | **No** | | **Yes** | | |
| Ca, mg/dl | 1759 | 8.56 ± 0.50 | 61 | 8.11 ± 0.51 | < 0.001 |
| CorrCa, mg/dl | 1448 | 8.80 ± 0.44 | 18 | 8.61 ± 0.40 | 0.06 |
| Mg, mg/dl | 813 | 2.06 ± 0.33 | 46 | 2.32 ± 0.34 | < 0.001 |
| P, mg/dl | 1232 | 3.26 ± 0.70 | 42 | 3.40 ± 1.17 | 0.455 |
| **Need for ICU admission** | | | | | |
| | **No** | | **Yes** | | |
| Ca, mg/dl | 1741 | 8.57 ± 0.50 | 79 | 8.15 ± 0.55 | < 0.001 |
| CorrCa, mg/dl | 1467 | 8.80 ± 0.44 | 29 | 8.64 ± 0.46 | 0.079 |
| Mg, mg/dl | 798 | 2.06 ± 0.32 | 61 | 2.27 ± 0.40 | < 0.001 |
| P, mg/dl | 1223 | 3.26 ± 0.70 | 51 | 3.37 ± 1.08 | 0.468 |
| **In-hospital mortality** | | | | | |
| | **No** | | **Yes** | | |
| Ca, mg/dl | 1655 | 8.57 ± 0.50 | 165 | 8.34 ± 0.55 | < 0.001 |
| CorrCa, mg/dl | 1340 | 8.79 ± 0.44 | 126 | 8.89 ± 0.48 | 0.020 |
| Mg, mg/dl | 776 | 2.07 ± 0.31 | 83 | 2.11 ± 0.48 | 0.467 |
| P, mg/dl | 1158 | 3.28 ± 0.71 | 116 | 3.18 ± 0.80 | 0.232 |
| **Composite** | | | | | |
| | **No** | | **Yes** | | |
| Ca, mg/dl | 1584 | 8.58 ± 0.49 | 236 | 8.30 ± 0.55 | < 0.001 |
| CorrCa, mg/dl | 1314 | 8.79 ± 0.43 | 152 | 8.85 ± 1.48 | 0.150 |
| Mg, mg/dl | 723 | 2.04 ± 0.30 | 136 | 2.17 ± 0.46 | 0.008 |
| P, mg/dl | 1113 | 3.27 ± 0.69 | 161 | 3.23 ± 0.88 | 0.518 |

Data are the mean ± SD. Ca: Calcium; CorrCa: Corrected calcium; Mg: Magnesium; P: Phosphorus; ICU: Intensive care unit.

ly related to all the studied poor outcome variables (p = 0.049 for mechanical ventilation; p = 0.004 for ICU admission; p < 0.001 for mortality and composite variable). Hypophosphatemia was significantly related to both mortality (p = 0.024) and composite variable (p = 0.009).

## Logistic regression analysis

Univariate and multivariate analysis for mechanical ventilation, ICU admission and mortality are shown in detail in **Table S7** (**Supplementary Material**). In brief, in the multivariate analysis, mechanical ventilation was negatively related with serum CorrCa levels [OR 0.19 (0.05–0.72); p = 0.014] and with the age of the patients [OR 0.88 (0.81–0.96); p = 0.005] (**Table S7A**). The need for ICU admission was also negatively related to CorrCa levels [OR 0.25 (0.09–0.66); p = 0.005] and the age of the subjects [OR 0.92 (0.87–0.98); p = 0.008], as well as positively with the presence of diabetes [OR 6.88 (1.21–36.77); p = 0.029] (**Table S7B**). Death during admission was positively related to the age of the patients [OR 1.14 (1.10–1.18); p < 0.001], and the presence of elevated creatinine [OR 2.12 (1.12–4.03); p = 0.021] and LDH levels [OR 2.37 (1.25–4.49); p = 0.008], and negatively to serum albumin levels [OR 0.28 (0.12–0.63); p = 0.002] (**Table S7C**).

In the multivariate analysis, the composite variable of poor outcome was negatively related to serum albumin levels [OR 0.08 (0.02–0.32); p < 0.001] and positively to the alteration of coagulation shown by elevation of the D-dimer [OR 3.64 (1.25–10.63); p = 0.018] (▶ **Table 3**). We did not find any significant relationship between CorrCa, Mg and P levels and the composite variable.

Lastly, ROC curve analysis showed that the predictive ability of CorrCa for the events studied was limited [AUC for mechanical ventilation, 0.64 (95 % CI, 0.50–0.77); AUC for ICU admission, 0.62 (95 % CI, 0.52–0.73)] (**Supplementary Material, Fig. S1**).

# Discussion

Results of the present paper have been collected in a sample of 2473 patients admitted for COVID-19 in a general hospital. In agreement with previous reports [8–12], our data show that most of the registered comorbidities and laboratory parameters were related to the poor outcome of the disease and to in-hospital mor-

▶ **Table 3** Results of univariate and multivariate logistic regression analysis to study the influence of several covariates on the composite variable of poor outcome.

| | Composite variable of poor outcome | | | | | |
|---|---|---|---|---|---|---|
| | Univariate analysis | | | Multivariate analysis | | |
| | OR | 95% CI | p | OR | 95% CI | p |
| CorrCa, mg/dl | 1.37 | 0.93–2.01 | 0.115 | | | |
| Mg, mg/dl | **2.68** | **1.54–4.68** | **0.001** | 1.13 | 0.33–3.86 | 0.847 |
| P, mg/dl | 0.91 | 0.72–1.15 | 0.439 | | | |
| Sex, male | 1.51 | 1.21–1.89 | <0.001 | 1.00 | 0.38–2.69 | 0.993 |
| Age, years | **1.05** | **1.04–1.05** | **<0.001** | 1.03 | 0.99–1.07 | 0.064 |
| Diabetes | **2.03** | **1.60–2.57** | **<0.001** | 1.51 | 0.60–3.80 | 0.288 |
| Obesity | 0.93 | 0.66–1.33 | 0.701 | | | |
| Hypertension | **1.97** | **1.60–2.43** | **<0.001** | 0.93 | 0.35–2.49 | 0.879 |
| CHD | **2.48** | **1.81–3.38** | **<0.001** | 2.72 | 0.76–9.77 | 0.124 |
| Cr > 0.76 mg/dl | **2.26** | **1.73–2.95** | **<0.001** | 1.17 | 0.46–2.98 | 0.749 |
| Lymphocytes, ≤ 1.04 × $10^3$/µl | **2.64** | **2.07–3.37** | **<0.001** | 1.42 | 0.58–3.45 | 0.442 |
| Albumin, g/dl | **0.10** | **0.07–0.16** | **<0.001** | **0.08** | **0.02–0.32** | **<0.001** |
| CRP, > 67.1 mg/l | **2.14** | **1.65–2.77** | **<0.001** | 0.56 | 0.22–1.42 | 0.222 |
| D–dimer, > 0.86 µg/ml | **3.10** | **2.44–3.93** | **<0.001** | **3.64** | **1.25–10.63** | **0.018** |
| LDH, > 267 U/l | **1.78** | **1.36–2.14** | **<0.001** | 1.13 | 0.48–2.63 | 0.785 |
| $SpO_2$, ≤ 93.3 % | **1.57** | **1.21–2.04** | **0.001** | 0.83 | 0.36–1.93 | 0.666 |

Multivariate model includes demographic, clinical and analytical variables with p < 0.10 in the univariate model. OR: Odds ratio; CI: Confidence interval; CorrCa: Corrected calcium; Mg: Magnesium; P: Phosphorus; CHD: Coronary heart disease; Cr: Creatinine; CRP: C-reactive protein; LDH: Lactate dehydrogenase; $SpO_2$: Arterial oxygen saturation; ICU: Intensive care unit. In the univariate and multivariate regressions, the sample size for the variables sex, age, diabetes, obesity, hypertension, and CHD is 2437 (whole cohort). The sample size is limited by the availability of data in the following variables: CorrCa, 1466; Mg, 859; P, 1274; Cr, 2005; lymphocytes, 2178; albumin, 1719; CRP, 2068; D-dimer, 2344; LDH, 1939; $SpO_2$, 1491. Bold values indicate statistically significant values.

tality. Importantly, our data also show that there are significant and relevant relationships between some Ca metabolism parameters and poor disease progression. In an initial analysis (▶ **Table 2**), we observed that patients with need for mechanical ventilation, ICU admission, in-hospital mortality or composite variable exhibited significantly lower total Ca values than patients without these characteristics. However, this was not the case when analyzing CorrCa. On the other side, hypermagnesemia was more common in patients with poor prognosis, while P values do not seem to exert any relevant influence on outcome or mortality.

Our multivariate analysis clearly showed that serum CorrCa was not related to in-hospital mortality or the composite variable. However, we found a significant negative relationship between serum CorrCa and both the need for mechanical ventilation and ICU admission. Nevertheless, we have to admit that the predictive value of this analytical parameter was weak, as shown by the result of the analysis of the ROC curves for mechanical ventilation and ICU admission. The positive relationships between Mg values and the need for mechanical ventilation, ICU admission or composite variable, were not confirmed in the multivariate analysis. This multivariate analysis has allowed us to confirm some well-known risk factors for poor prognosis, such as age, diabetes, hypoalbuminemia, or elevated creatinine, LDH, or D-dimer levels [9, 11, 12, 15]. Age, elevated creatinine and LDH levels, and decreased albumin levels were other factors with an independent association with mortality. The decrease in albumin and the elevation of D-dimer were significantly associated with the composite variable. To the best of our knowledge, this is the first study that analyzes the prognostic value of the three main parameters of Ca metabolism in a large sample of unselected patients, consecutively admitted for COVID-19 in a general hospital. Nevertheless, due to the retrospective nature and the methodological limitations of our study, we must be cautious with the interpretations that we can give to our data and the mechanisms that can be adduced to explain the findings.

Studies prior to the current pandemic revealed that hypocalcemia is common among critically ill patients [19, 20], and that a correlation exists between hypocalcemia and poor clinical outcome in critically ill patients [21–24]. In patients with COVID-19, the initial study by di Filippo et al. [8] showed that serum Ca concentrations were negatively related to LDH and CRP levels and that hypocalcemia was a risk factor for hospital admission. Another retrospective study compared 420 subjects with a positive result in RT-PCR for

SARS-CoV-2, with 165 patients with similar symptoms, but negative for this test. It was observed that the former presented significantly reduced levels of total and ionized Ca levels [25]. However, unlike ours, these studies were conducted in patients who attended the emergency department.

A few studies have addressed the significance of Ca levels as a predictor of disease severity in hospitalized patients. Two studies, one including 125 patients [11] and another 445 patients [26], showed that hypocalcemia was a risk factor associated with longer hospitalization duration in patients with COVID-19. However, there was no data on ICU admission or mortality in the former [11] and there was no association between hypocalcemia and mortality in the latter [26]. The study by Liu et al. [9], in 107 patients, showed that CorrCa levels are associated with a poor outcome evaluated by a composite variable that included the need for mechanical ventilation, ICU admission or death, but they do not provide data on the individual components of the composite variable. Other studies have linked low Ca levels with pro-inflammatory markers, multiple organ injuries and the severity of the disease [10, 27–31].

In a study of 241 admitted patients, Sun et al. [10] reported an association between hypocalcemia and 28-day mortality. However, this report used non corrected Ca levels, and shows a high mortality (23.3 %) only in patients with severe hypocalcemia (< 8.0 mg/dl), but an absence of mortality in subjects with less marked degree of hypocalcemia (8–8.8 mg/dl). Subsequently, in a group of 91 patients with COVID-19, the rates of death and ICU admission were found to be significantly higher among subjects with hypocalcemia [32]. Torres et al. [27] evaluated 316 hospitalized patients and reported that patients with hypocalcemia were admitted to the ICU more frequently than subjects with normal Ca. However, no differences in mortality were observed. Hypocalcemia was significantly associated with in-hospital mortality in a study including 120 patients, although this study was limited to severe cases of COVID-19 admitted at an anesthesia department [33]. Lastly, a recent meta-analysis [34] including 2032 patients from 7 studies showed that serum Ca was lower in patients with poor outcome, defined as a composite of mortality and severity, with an OR of 3.19 (95 % CI, 2.02–5.06). However, this study was limited by the small number of studies included and the limited power of the meta-regression analysis.

In short, the few studies that have assessed the relationship between calcemia and mortality or poor outcome have been carried out in limited cohorts of patients, and the obtained results have been highly variable. The observed discrepancies might be accounted for by differences in sample size, epidemiology of the disease, degree of severity of admitted patients, level of complexity of the centers, local protocols, available therapies, restrictive hospital admission policies or definitions of hypocalcemia. We think that, by and large, our results are in line with what has been reported to date and provide a significantly larger sample size.

Very few studies have evaluated Mg and P levels. Our initial analysis suggested that elevated Mg levels were related to a poor outcome. However, in the multivariate analysis this significant relationship was not maintained. Serum P seemed to behave in a neutral way in relation to mortality or poor outcome. We have only found two studies with quantification of Mg or P levels [27, 35]. Torres et al. [27] observed hypomagnesemia [< 2.16 mg/dl) in 17 % of a cohort of 316 hospitalized patients but found no relationship with poor outcome or mortality. Pal et al. [35] compared the biochemical results of a group of 72 patients admitted with non-severe COVID-19 with 72 matched healthy controls. Ca, CorrCa and P levels were significantly lower in patients. No comparison was made with Mg levels. In this study, only 9 patients had moderate disease, while the rest had mild disease.

The causes of hypocalcemia in patients with severe COVID-19 have not been fully clarified. Several mechanisms have been suggested, including decreased dietary intake, alterations in intestinal absorption, hypoproteinemia, vitamin D deficiency, drug interactions, and imbalance in regulatory mechanisms involving PTH and vitamin D [13, 23]. A direct effect caused by SARS-CoV-2 has also been suggested. In fact, Ca plays a crucial role in the viral fusion of various enveloped viruses such as SARS-CoV, MERS-CoV, and Ebolavirus [3–5]. Ca ions might play a pivotal role in membrane entry and fusion of coronavirus via the Ca binding pocket [4]. According to this, a lower Ca concentration might reflect a higher viral load when the human body is infected with a coronavirus, leading to a prolonged period of viral shedding.

Proinflammatory cytokines in COVID-19 can inhibit PTH secretion and alter the response to PTH, producing imbalance of Ca [9, 36]. Vitamin D deficiency has been reported to be highly prevalent in COVID-19 patients [9, 10, 29, 33, 35] and is known to alter Ca metabolism, reducing intestinal absorption of Ca and P. It has been hypothesized that vitamin D deficiency may predispose to SARS-CoV-2 infection and to increased severity of COVID-19 [13, 37]. Furthermore, unbound and unsaturated fatty acids, which are elevated in patients with COVID-19, can bind to Ca and cause significant acute hypocalcemia [37, 38]. They can also induce cytokine storm and multiorgan system failure [39]. In contrast to Ca, Mg is not tightly regulated by hormones such as PTH or vitamin D. Thus, it is difficult to explain the possible association of hypermagnesemia with a poor prognosis of COVID-19, as suggested by our data in the univariate analysis. It might be speculated that the increase in cytokines caused by SARS-CoV2 could generate an increase in serum Mg in some patients due to interference with its renal elimination or to an effect on Mg release from bone deposits, but we do not have studies addressed to this issue.

Our study opens the question of why hypocalcemia is associated with a poor prognosis in patients with COVID-19. Different mechanisms have been proposed. Hypocalcemia can have a negative impact on heart function and may be lethal when severe and acute. In critically ill patients, hypocalcemia has been shown to be associated with prolonged ICU stay and increased mortality [40]. Ca has also been reported to be related to the lung function and capacity of defense against invading pathogenic microorganisms in pulmonary infections [41], suggesting that Ca imbalance might induce a delayed recovery from infections.

We acknowledge limitations in our study. Owing to the retrospective design, not all laboratory tests were available in all patients. Hence, the role of these missing indicators might be underreported in the prediction of long-term hospitalization. The relatively low number of patients with Mg determination has limited the statistical power of our study and may have induced a selection bias in those patients in whom the doctor requested a Mg determination. The treatments for COVID-19 in this study were not the

same for all patients throughout the year, as treatment protocols changed as knowledge of the disease advanced. Our study is a single-center study, and the data are from the year 2020. It is therefore possible that our results may not be extrapolated to other settings with different patient profiles or health policies, or that they have application to current patients or patients in the years to come. We have tried to include many confounding factors in our multivariate analysis, but it is possible that unidentified risk factors may play a significant role. We did not have the opportunity to assess serum levels of ionized Ca, vitamin D or PTH, as this is an observational study of clinical practice, and these data were not available. On the other hand, it was out of our objective to analyze calcium metabolism disorders in patients hospitalized for causes other than COVID-19, so we cannot compare our results with a control group. Nevertheless, our main strengths are the large sample size, including a huge cohort without exclusions, as well as the evaluation of serum Mg and P values.

In conclusion, our data show that serum CorrCa levels are negatively and significantly related to a poor outcome of COVID-19 manifested by need for mechanical ventilation and ICU admission, but not to in-hospital mortality. So far, there are no reports showing that the treatment of hypocalcemia can improve prognosis in hypocalcemic individuals with COVID-19. However, there are very recent encouraging data that an intervention on Ca metabolism with vitamin D supplements [42, 43] can substantially improve the prognosis and survival of these patients. Therefore, due to the persistence of the pandemic, the high number of ICU admissions, the lack of effective treatments in critically ill patients, and the increasing consumption of healthcare resources, our results suggest that CorrCa, an analyte that can be determined quickly and cheaply in the clinical laboratory, appears to behave as a marker of aggressiveness of COVID-19, and that early identification and correction of Ca metabolism derangements of these patients might be of substantial benefit.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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