J Nutr Health Aging. 2021;25(10):1140-1144
Published online October 20, 2021, http://dx.doi.org/10.1007/s12603-021-1690-7

Brief Report

Hypercalcemia as a Biomarker of Poor Prognosis in Frail Elderly Patients with COVID-19

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Abstract

The objective of this cohort study was to determine whether hypercalcemia in early COVID-19 was associated with 3-month mortality in frail elderly patients. Circulating calcium and albumin concentrations at hospital admission and 3-month mortality were assessed in geriatric patients hospitalized for COVID-19 with normal-to-high calcium concentrations. Hypercalcemia was defined as corrected calcium >2.5mmol/L. Covariables were age, sex, functional abilities, malignancies, hypertension, cardiomyopathy, number of acute health issues, use antibiotics and respiratory treatments. In total, 94 participants (mean±SD 88.0±5.5years; 47.9% women; 22.3% hypercalcemia; 0% hypocalcemia) were included. Sixty-five participants who survived at 3months exhibited less often hypercalcemia at baseline than the others (13.9% versus 41.4%, P=0.003). Hypercalcemia was associated with 3-month mortality (fully-adjusted HR=3.03, P=0.009) with specificity=0.86 and sensitivity=0.41. Those with hypercalcemia had shorter survival time than those with normocalcemia (log-rank P=0.002). In conclusion, hypercalcemia was associated with poorer survival in hospitalized frail elderly COVID-19 patients.

Key words: COVID-19, SARS-CoV-2, calcium, biomarker, prognosis, older adults.

Introduction

COVID-19, the identification of which dates from the end of 2019, quickly became pandemic in the first months of 2020, affecting millions of people and causing hundreds of thousands deaths (1). The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects all population categories, with poorer prognosis in those exhibiting criteria of severity such as older age or a high number of comorbidities (2). Various biological agents have been examined in relation with COVID-19 prognosis, in particular those likely to increase the infection and inflammation risks.

In viral infections, the calcium is essential for virus structure formation, entry, gene expression, virion maturation and release (3), as evidenced by its increased consumption during COVID-19 (4). Notably, experimentation reports that SARS-CoV E gene encodes a small transmembrane protein with ion channel permeable to Ca2+, that is highly synthesized during infection. Consequent alterations of calcium homeostasis could promote the activation of inflammatory pathways leading to increased levels of IL-1β, TNF and IL-6 (5, 6). In this view, calcium status has been the matter of early attention to determine whether altered calcium concentrations were associated with poorer prognosis in COVID-19 (4, 7, 8). The first results showed that, while deep hypocalcemia was inversely correlated with COVID-19 severity, no conclusion could be reached about a possible link between hypercalcemia and the prognosis of COVID-19 (4, 7, 8). Hypercalcemia is, however, a recognized factor of poor prognosis in several other inflammatory diseases, in particular malignancies (9), and has also been previously associated with the onset of acute respiratory distress syndrome (ARDS), one of the main complications of COVID-19 (10, 11).

Calcium is present in two distinct forms in the plasma. The first one corresponds to ionized calcium which has an electric charge; the second form, non-ionized, binds to plasma albumin. Thus, in the event of hypoalbuminemia, the measured calcium concentration may be falsely altered, which encourages the application of a formula to correct calcium concentration. As hypoalbuminemia is particularly frequent during COVID-19 (12), the link (if any) between hypercalcemia and COVID-19 prognosis should be examined by using the corrected calcium concentration, which was not the case in previous reports. The aim of the present longitudinal cohort study was to determine whether hypercalcemia, defined as an elevated corrected calcium concentration in early COVID-19, was associated with 3-month mortality in frail elderly patients with COVID-19.

Methods

The GERIA-COVID study is a longitudinal observational study conducted in the geriatric acute care unit dedicated to COVID-19 patients in the University Hospital of Angers, France, during the first wave of the COVID-19 pandemic (ClinicalTrials.gov NCT04560608). Data of the GERIA-COVID study were retrospectively collected from patients' records.

Received May 4, 2021
Accepted for publication May 18, 2021

Study population

The inclusion criteria in the GERIA-COVID study were as follows: 1) patients aged 75 years and over hospitalized in the geriatric acute care unit of Angers University Hospital, France, in March-June 2020; 2) no objection from the patient and/or relatives to the use of anonymized clinical and biological data for research purpose.

The inclusion criteria for the present analysis were as follows: 1) COVID-19 diagnosed with RT-PCR and/or chest CT-scan; 2) data available on the serum calcium and albumin concentrations at hospital admission; 3) no hypocalcemia, defined as corrected calcium concentration ≤1.9mmol/L; 4) data available on the vital status 3 months after the diagnosis of COVID-19. Ninety-seven patients were consecutively diagnosed with COVID-19 during the study period in the unit and were recruited in the GERIA-COVID study. Among them, three participants had missing values of serum calcium or albumin concentrations. All participants had normal-to-high calcium concentration. Finally, 94 participants were included in the present analysis.

Hypercalcemia

Serum calcium concentration was measured in mmol/L (ADVIA Chemistry Calcium_2, Bayer Healthcare AG, Leverkusen, Germany) locally at the laboratory of the University Hospital of Angers, France, in all patients on the day of hospital admission for COVID-19. The albuminemia was measured in g/L on the same blood sample to correct the calcium concentration according to the following formula: Corrected calcium concentration mmol/L = measured calcium concentration mmol/L - 0.025 x (serum albumin concentration g/L - 40). Hypercalcemia was consensually defined as corrected calcium concentration > 2.5 mmol/L.

Overall mortality

The main outcome was the 3-month all-cause mortality. Follow-up started from the day of COVID-19 diagnosis for each patient and continued for 3 months or until death when applicable. Vital status was recovered by contacting the patients and their relatives by telephone, and by monitoring the National Institute of Statistics and Economic Studies (INSEE) register (https://www.insee.fr/fr/information/4190491).

Covariables

Potential confounders were age, sex, functional abilities, history of malignancies, hypertension, cardiomyopathy, number of acute health issues at hospital admission, hospital use of antibiotics and pharmacological treatments of respiratory disorders. Functional abilities prior to COVID-19 were measured from 1 to 6 (best) with the Iso-Resources Groups (GIR) (13). History of hematological and solid malignancies, of hypertension and of cardiomyopathy were noted from the medical register, and by interviewing patients, their relatives and family physicians. Acute health issues were defined as diseases with sudden onset and rapid progression, whatever their nature or site. The use of antibiotics (i.e., quinolones, betalactams, sulfonamides, macrolides, lincosamides, aminoglycosides, among others), and/or pharmacological treatments of respiratory disorders (i.e., beta2-adrenergic agonists, inhaled corticosteroids, antihistamines, among others) were noted from prescriptions during hospitalization.

Statistical analysis

The participants' characteristics were summarized using means and standard deviations (SD) or frequencies and percentages, as appropriate. Firstly, comparisons between participants separated according to the vital status at the end of month 3 of the diagnosis of COVID-19 were performed using Chi-square test (or Fisher exact test) or Student t test (or Mann-Whitney Wilcoxon test according to the normality assessment), as appropriate. Secondly, a partially-adjusted (accounting for age, gender, and GIR score) and a fully-adjusted Cox regression were used to examine the associations of hypercalcemia (independent variable) with the 3-month mortality (dependent variable). The models produce a survival function that provides the probability of death at a given time for the characteristics supplied for the independent variables. Finally, the elapsed time to death was studied by survival curves computed according to Kaplan-Meier method and compared by log-rank test. P-values<0.05 were considered significant. All statistics were performed using SAS® version 9.4 software (Sas Institute Inc) and R (R core Team, 2018).

Ethics

The study was conducted in accordance with the ethical standards set forth in the Helsinki Declaration (1983). No participant or relatives objected to the use of anonymized clinical and biological data for research purposes. Ethics approval was obtained from the Ethics Board of the University Hospital of Angers, France (2020/100). The study protocol was also declared to the National Commission for Information Technology and civil Liberties (CNIL; ar20-0087v0).

Results

Ninety-four participants (mean±standard deviation, 88.0±5.5years; 47.9% women; 22.3% hypercalcemia; 0% hypocalcemia) were included in the analysis. Sixty-five participants survived COVID-19 after 3 months of follow-up while 29 died.

Table 1 shows the characteristics of the participants separated according to their 3-month survival. Hypercalcemia was significantly more frequent in the group who died during the 3 months (41.4% versus 13.9%, P=0.003). This group was also more disabled (P=0.01), had more often a history of malignancies (P=0.004), had a greater number of health issues on admission (P=0.02) and used antibiotics more often during

Table 1. COVID-19 patients' characteristics at baseline according to 3-month mortality, and multiple Cox proportional-hazards models showing the hazard ratio for 3-month all-cause mortality (dependent variable) according to corrected hypercalcemia at baseline (independent variable) (n=94)

Characteristics at baseline	Total cohort (n=94)	3-month mortality		P-value*	Multiple Cox proportional-hazards models			
		No (n=65)	Yes (n=29)		Unadjusted model		Fully-adjusted model	
					HR [95%CI]	P-value	HR [95%CI]	P-value
Demographical data								
Age (years)	88.0±5.5	87.9±5.4	88.3±5.8	0.748	1.01 [0.95;1.09]	0.678	1.02 [0.94;1.11]	0.629
Female gender	45 (47.9)	35 (53.9)	10 (34.5)	0.083	0.55 [0.26;1.18]	0.124	0.53 [0.22; 1.28]	0.155
GIR score (/6)	4 [2;4]	4 [3;5]	3 [2;4]	0.013	0.70 [0.54;0.93]	0.012	0.84 [0.61;1.16]	0.280
Comorbidities								
Hematological and solid malignancies	32 (34.0)	16 (24.6)	16 (55.2)	0.004	3.01 [1.47;6.36]	0.003	3.40 [1.55;7.58]	0.003
Hypertension	59 (62.8)	41 (63.1)	18 (62.1)	0.926	0.98 [0.46;2.08]	0.963	1.30 [0.55;3.08]	0.554
Cardiomyopathy	49 (52.1)	33 (50.8)	16 (55.2)	0.693	1.17 [0.56;2.43]	0.676	1.21 [0.53;2.77]	0.647
Hospitalization								
Hypercalcemia†	21 (22.3)	9 (13.9)	12 (41.4)	0.003	3.00 [1.43;6.28]	0.004	3.03 [1.32;6.93]	0.009
Number of acute health issues at hospital admission	3 [2;4]	2 [1;4]	3 [2;5]	0.019	1.30 [1.04;1.63]	0.021	1.26 [0.96;1.65]	0.097
Use of antibiotics‡	63 (67.0)	39 (60.0)	24 (82.8)	0.030	2.63 [1.00;6.90]	0.049	2.51 [0.86;7.29]	0.092
Use of pharmacological treatments of respiratory disorders‖	11 (11.7)	7 (10.8)	4 (13.8)	0.733	1.37 [0.48;3.95]	0.556	2.17 [0.67;7.06]	0.197

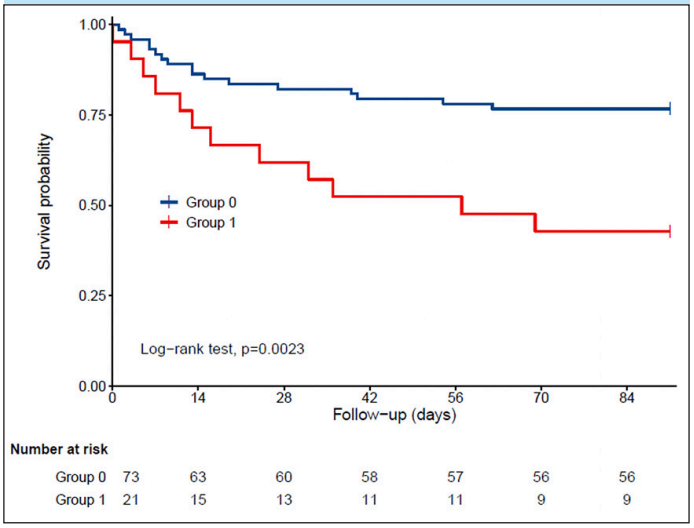
Data presented as median [interquartile range] or n (%), as appropriate; CI: confidence interval; COVID-19: Coronavirus Disease 2019; GIR: Iso Resource Groups; HR: hazard ratio; IQR: interquartile range; *: between-group comparisons based on Chi-square test (or Fisher exact test) or Student t test (or Mann-Whitney Wilcoxon test according to the normality assessment), as appropriate; †: corrected calcium concentration > 2.5 mmol/L; ‡: quinolones, beta-lactams, sulfonamides, macrolides, lincosamides, aminoglycosides, among others; ‖: beta2-adrenergic agonists, inhaled corticosteroids, antihistamines, among others.

hospitalization (P=0.03) than the others.

In Cox proportional-hazards models, hypercalcemia was directly associated with 3-month mortality in frail elderly patients with COVID-19 (Table 1). Using "no hypercalcemia" as a reference (hazard ratio (HR)=1), the HR for mortality in participants with hypercalcemia was 3.00 [95% confidence interval (CI):1.43-6.28] (P=0.004) in the unadjusted model, HR=2.96 [95%CI:1.38-6.35] (P=0.005) after partial adjustment, and HR=3.03 [95%CI:1.32;6.93] (P=0.009) after adjusting for all potential confounders. The specificity for predicting death from hypercalcemia was high at 86.2%, but relatively less sensitive at 41.4%. The history of malignancies was also associated with 3-month mortality (HR=3.40 with P=0.003). Of note, no association of hypercalcemia with 3-month mortality was retrieved in the 32 participants with a history of malignancies (HR=2.2 [95%CI:1.32;6.93]).

Finally, Kaplan-Meier distributions showed in Figure 1 that COVID-19 patients with hypercalcemia had shorter survival time than those with normocalcemia (log-rank P=0.002).

Figure 1. Kaplan-Meier estimates of the cumulative probability of COVID-19 participants' survival according to corrected hypercalcemia (n=94)



Group 0: No hypercalcemia; Group 1: Hypercalcemia, defined as corrected calcium concentration > 2.5 mmol/L

Discussion

The main result of this cohort study is that, irrespective of all measured potential confounders, hypercalcemia estimated in early COVID-19 from corrected calcium concentration was associated with higher 3-month mortality in frail elderly patients with COVID-19 and normal-to-high calcium concentrations. This novel finding calls for particular attention to the calcium status in patients with COVID-19. Further studies are needed to determine whether the normalization of hypercalcemia is associated with improved COVID-19 survival.

The association between serum calcium concentration and COVID-19 prognosis has been the subject of some previous studies, all of them using the measured but uncorrected calcium value. Yang and al. found, in 226 suspected and confirmed patients with COVID-19, no association between calcium concentrations >2.52mmol/L and severe-to-critical forms of COVID-19 (P=0.422), while calcium concentrations <2.11mmol/L were instead associated with more frequent severe-to-critical forms (OR=15, P=0.018) (14). Similarly, Sun et al. also reported greater 28-day mortality risk in 43

patients with COVID-19 and calcium concentration ≤2mmol/L compared to those with COVID-19 and normocalcemia (7). Compared to our analysis, these previous studies did not account for albuminemia. Thus we provide here, to the best of our knowledge, the first evidence that hypercalcemia estimated from corrected calcium concentration is able to predict the 3-month mortality risk in frail elderly patients with COVID-19 and normal-to-high calcium concentrations.

This result was not specific of patients with a history of malignancies, and the specificity for the prediction of death from hypercalcemia was much greater than its sensitivity. This suggests a fairly direct role for hypercalcemia in the poor prognosis of COVID-19, and not just related to the conditions causing hypercalcemia. Calcium plays a central role for viral invasion. By infecting cells using a replication system, SARS-CoV-2 modifies proteins on the cell membrane, such as the addition of viroporins, a small transmembrane protein. Viroporins have ion non-selective channel activity for Ca2+, thereby causing a dysregulation of calcium homeostasis (15). The alteration of calcium homeostasis within cells stimulates the activation of the nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome, which results in the overproduction of IL-1β (6). IL-1β is a pro-inflammatory cytokine that plays a crucial role in resolving infectious processes. In contrast, its overproduction has been correlated with severe inflammatory diseases and lung injuries such as ARDS (16,17,18, 19), a lethal condition induced by SARS-CoV-2.

Some limitations should be acknowledged. First, this study was single-centered and participants restricted to frail elderly patients hospitalized for COVID-19 with normal-to-high calcium concentrations who might be unrepresentative of all elderly people. Second, causes other than malignancies can be proposed to explain hypercalcemia but have not been explored here, such as for example the sedentary lifestyle linked to the national lockdown (20). Third, although we were able to control for important characteristics that could modify the association, residual potential confounders might still be present such as the history of chronic renal failure. Fourth, the observational design of our study was less robust than an interventional study and precluded causal inference.

Conclusions

In conclusion, we found that, irrespective of all measured potential confounders, hypercalcemia estimated in early COVID-19 from corrected calcium concentration was associated with higher 3-month mortality in frail elderly patients with normal-to-high calcium concentration. Biomarkers' clinical usefulness is defined as their capability to influence clinicians to diagnose the disease, predict prognosis, and guide treatment. Here, hypercalcemia is certainly not credible as a diagnostic marker of COVID-19 but appears as a specific prognostic marker for severe forms of COVID-19. Its ability to predict life-threatening risk in COVID-19 patients should be further explored on larger cohorts including a variety of ages and populations with different health conditions. Finally, intervention studies aimed at normalizing serum calcium are required to determine whether the correction of hypercalcemia improves survival in COVID-19.

Acknowledgments: The authors wish to thank the GERIA-COVID study group. GERIA-COVID study group: Cédric Annweiler1, Marine Asfar1, Mélinda Beaudenon1, Jean Barré1, Antoine Brangier1, Mathieu Corvaisier1, Guillaume Duval1, Jennifer Gautier1, Mialy Guenet1, Jocelyne Loison1, Frédéric Noublanche1, Marie Otekpo1, Hélène Rivière1, Guillaume Sacco1, Romain Simon1. Affiliations: 1: Department of Geriatric Medicine, University Hospital, Angers, France. The authors have listed everyone who contributed significantly to the work in the Acknowledgments section. Permission has been obtained from all persons named in the Acknowledgments section. There was no compensation for this contribution.

Conflict of interest statement: All authors declare they do not have any other financial and personal conflicts of interest with this manuscript.

Sponsor's role: None.

Authors contribution: - CA has full access to all of the data in the study, takes responsibility for the data, the analyses and interpretation and has the right to publish any and all data, separate and apart from the attitudes of the sponsors. All authors have read and approved the manuscript. - Study concept and design: CA. - Acquisition of data: DP, GD, MO, MA, JG and CA. - Analysis and interpretation of data: DP, JG and CA. - Drafting of the manuscript: CA and DP. - Critical revision of the manuscript for important intellectual content: GD, MO, MA and JG. - Obtained funding: Not applicable. - Statistical expertise: JG. - Administrative, technical, or material support: CA. - Study supervision: CA.

Data availability: Patient level data are freely available from the corresponding author at Cedric.Annweiler@chu-angers.fr. There is no personal identification risk within this anonymized raw data, which is available after notification and authorization of the competent authorities.

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How to cite this article: D. Pamart, M. Otekpo, M. Asfar, et al. Hypercalcemia as a Biomarker of Poor Prognosis in Frail Elderly Patients with COVID-19. J Nutr Health Aging. 2021;25(10):1140-1144, http://dx.doi.org/10.1007/s12603-021-1690-7