Letter to the Editor

Fabrizio Cappellini*, Rinaldo Brivio, Marco Casati, Annalisa Cavallero, Ernesto Contro and Paolo Brambilla

Low levels of total and ionized calcium in blood of COVID-19 patients

https://doi.org/10.1515/cclm-2020-0611
Received April 28, 2020; accepted May 13, 2020

Keywords: calcium; COVID-19; ionized calcium; pandemic; SARS-CoV-2.

To the Editor,

As of April 27, 2020, the SARS-CoV-2 pandemic caused worldwide more than 3 million cases with more than 200,000 confirmed deaths. Italy, following USA and followed by Spain, is the country with a higher number of deaths, more than 26,000, with most of them occurring in Lombardia region [1]. Worldwide, hospital organizations have been deeply changed in the recent months; San Gerardo Hospital (Monza, Italy) increased its intensive care unit bed availability by almost 300% and a total of 20 wards were readdressed for the care of COVID-19 patients.

Patients admitted to the emergency department (ED) with clinical signs and symptoms suggestive of COVID-19 have been investigated with nasopharyngeal swabs for SARS-CoV-2 (RT-qPCR) plus biochemistry, hematology and coagulation test panels. Overall, we found an increase in patients who had a reduction in serum total calcium (TCa, mmol/L) and whole blood actual ionized calcium (Ca^{2+}, mmol/L) levels. Therefore, we retrospectively evaluated TCa and Ca^{2+} in consecutive adult patients admitted to the ED during the period February 27th–March 30th and suspected to be positive for SARS-CoV-2 after evaluation of anamnestic data, and physical and diagnostic examination made by the ED clinicians. We took into consideration all cases for whom the swab had been performed on the same day of admission to ED, and TCa and Ca^{2+} had been measured (within 30 min for TCa and immediately for Ca^{2+}), on samples obtained during the first ED examination. The aim was to give a realistic picture of the calcium measurements according to subsequent stratification of subjects in positive or negative to infection by the results of the nasopharyngeal swabs. The study population included 585 patients: 386 males and 199 females, 420 positive and 165 negative to SARS-CoV-2, with mean and median age equal to 66 years (range 18–101 years).

TCa was measured photometrically (Cobas c702, Roche Diagnostics, Mannheim, Germany) on serum obtained after centrifugation of whole blood (10 min at $3000 \times g$ at room temperature) collected in plastic tubes with gel separator and clot activator (SST II ADVANCE, REF 366881 Becton Dickinson). Ca^{2+} was measured by direct potentiometry (RP500, Siemens Healthcare, Erlangen, Germany) on arterial blood sampling syringes with dry lithium heparin (Smiths Medical – Portex, REF 4043E). Cumulative frequency distribution plot revealed that COVID-19 patients had lower TCa (Figure 1A) and Ca^{2+} (Figure 1B) concentrations with respect to non-COVID-19 patients. TCa medians and interquartile ranges for COVID-19 and non-COVID-19 patients were 2.15 (2.05–2.23) and 2.28 (2.15–2.38), respectively, and for Ca^{2+} were 1.12 (1.09–1.15) and 1.17 (1.13–1.21), respectively. The Mann-Whitney test was performed to compare medians that resulted to be statistically different, $p < 0.0001$. Given that we observed a decrease for TCa and Ca^{2+} with increasing age and lower values in males with respect to females, we performed a multiple regression analysis in order to test if: (a) positivity to SARS-Cov-2, (b) age and (c) gender of patients could independently affect TCa and Ca^{2+}. For TCa, the SARS-CoV-2 positivity (coefficient: −0.1247, $p < 0.0001$), age (coefficient: −0.0015, $p = 0.0001$) and male gender (coefficient: −0.0337, $p = 0.0039$) significantly and negatively affected the analyte concentration; for Ca^{2+}, the

***Corresponding author: Fabrizio Cappellini,** University Department of Laboratory Medicine, University Milano-Bicocca, ASST Monza, San Gerardo Hospital, Via G.B. Pergolesi 33, 20900 Monza (MB), Italy, Phone: 0039 039 2336903, E-mail: fabrizio.cappe.lavoro@gmail.com. https://orcid.org/0000-0002-5517-1021
Rinaldo Brivio, Marco Casati and Paolo Brambilla: University Department of Laboratory Medicine, University Milano-Bicocca, ASST Monza, San Gerardo Hospital, Monza (MB), Italy
Annalisa Cavallero: Laboratory of Microbiology, ASST Monza, San Gerardo Hospital, Monza (MB), Italy
Ernesto Contro: Emergency Department, ASST Monza, San Gerardo Hospital, Monza (MB), Italy

Published online May 27, 2020

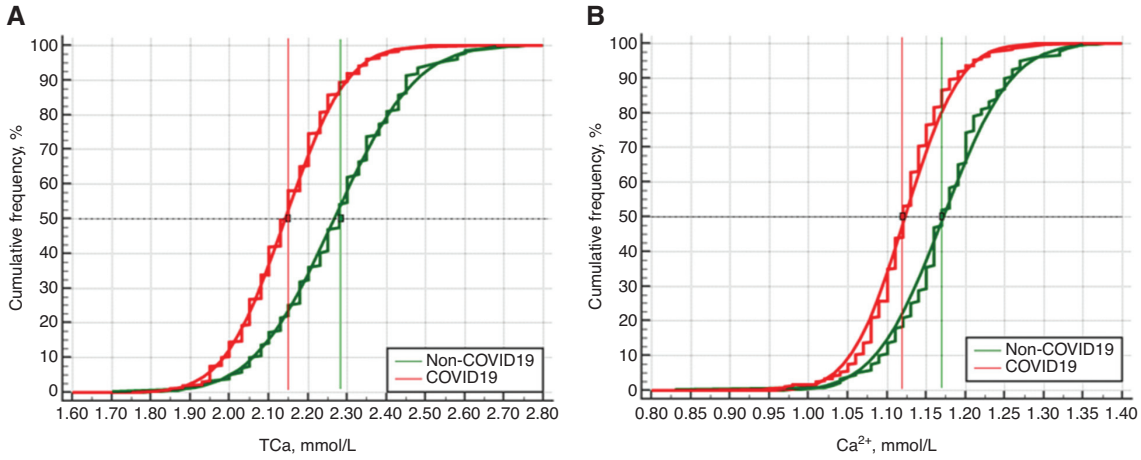

Figure 1: Calcium values distribution in the study population.
Cumulative frequency distribution plot of TCa (A) and Ca^{2+} (B) in COVID-19 and non-COVID-19 patients admitted to ED with clinical suspect of SARS-CoV-2 infection. Red and green rectangles correspond to the median TCa and Ca^{2+} values observed in COVID-19 and non-COVID-19 patients, respectively.

SARS-CoV-2 positivity (coefficient: −0.0486 $p < 0.0001$), but not age ($p = 0.0747$) and gender ($p = 0.0895$), affected the analyte concentration. We performed an analysis of covariance (ANCOVA) with the aim to compare TCa and Ca^{2+} means in COVID-19 and non-COVID-19 adjusting for age and gender. In COVID-19, TCa was equal to 2.14 (95% CI 2.13–2.15) and Ca^{2+} was equal to 1.12 (95% CI 1.12–1.13). In non-COVID-19, TCa was equal to 2.27 (95% CI 2.25–2.29) and Ca^{2+} was equal to 1.17 (1.16–1.18). Both for TCa and Ca^{2+}, the differences between groups are statistically significant, $p < 0.0001$. Statistical analyses were performed using MedCalc for Windows (version 18.11.6) on an anonymized dataset which did not require the review of the Ethics Committee.

To the best of our knowledge, these findings are unique in published literature; many studies [2–5] reported abnormalities in laboratory tests of COVID-19 patients (decrease in lymphocyte count, platelet count and albumin, and increase in neutrophil count, LDH, ALT, AST, total bilirubin, creatinine, cardiac troponins, D-dimer, PT, procalcitonin and CRP), but calcium alterations have not yet been observed. We evaluated blood pH for all subjects in order to test the chance that the Ca^{2+} reduction could be caused by a pH increase in COVID-19 patients consequent to hyperventilation. We would rule out this possibility because (a) the mean pH values in COVID-19 and non-COVID-19 groups are similar (7.43 and 7.47, respectively), and (b) we have repeated ANCOVA adding pH as covariate observing that the difference in Ca^{2+} between groups still remains statistically significant ($p < 0.0001$). Moreover, we found the same results for TCa and Ca^{2+} leading us to think that the reduction observed is independent of the albumin decrease seen in COVID-19 patients.

Serum calcium modifications may be due to alterations in intestinal absorption, imbalance in regulatory mechanism involving PTH and D-Vitamin, or a direct effect caused by SARS-CoV-2. In viral infections, Ca^{2+} is essential for virus structure formation, entry, gene expression, virion maturation and release [6]. Experiments *in vitro* and in animal models infected with SARS-CoV demonstrated that SARS-CoV *E* gene encodes a small transmembrane protein with ion channel activity that is highly synthesized during infection and that is mainly localized in the Golgi apparatus and endoplasmic reticulum Golgi apparatus intermediate compartment (ERGIC). These ion channels are permeable to Ca^{2+}, and it was demonstrated that calcium homeostasis alteration within the cell could promote the activation of inflammatory pathways leading to increased levels of IL-1b, TNF and IL-6, linked to lung cell damage and edema accumulation [7, 8]. Given the high similarity between SARS-CoV-2 and SARS-CoV genomes, it is likely that the mechanisms seen in SARS-CoV could be the same for SARS-CoV-2. For this reason, our observation could be a direct consequence of a known alteration of intracellular calcium homeostasis used by the virus as a strategy in its favor.

Further studies should be performed with the aim to confirm our findings, also taking into account all the factors that could affect calcium homeostasis, and to evaluate if calcium measurements can be used as a prognostic and/or severity marker in patients with SARS-CoV-2 infection.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Employment or leadership: None declared.
Honorarium: None declared.
Research funding: None declared.
Competing interests: Authors state no conflict of interest.
Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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