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LETTER TO THE EDITOR

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Pre-analytical and analytical procedures to avoid loss of vitamin C. Comment on: "COVID-19: up to 82% critically ill patients had low vitamin C values"

Luis Chiscano-Camón[1,2,3], Juan Carlos Ruiz-Rodriguez[1,2,3*], Roser Ferrer[4], Sílvia Camós[4], Adolf Ruiz-Sanmartin[1,2,3] and Ricard Ferrer[1,2,3]

Main text

We have read with great interest the short report by Tomasa – Irriguible et al. about the vitamin C levels in critically ill COVID-19 adult patients with ARDS [1]. It refers to our study, in which it was found that in patients with ARDS caused by COVID-19, vitamin C levels were extremely low [2]. However, the results of our study are questioned on the basis that according to Tomasa – Irriguible et al., the veracity of the methodology used to assess the vitamin C status of the patients was not clear, so the values could be artifactually low.

According to the literature, vitamin C is a very unstable water-soluble micronutrient which can be easily oxidized or hydrolyzed [3]. We agree with the authors that the determination of vitamin C levels requires very rigorous pre-analytical and analytical procedures to avoid loss of vitamin. In our study we followed the recommendations published by Pullar et al. [4] and we performed the pre-analytical phase with great care.

In our case, venous blood was collected into a 4 mL vacuette tube with anticoagulant lithium heparin and immediately delivered to the laboratory protected from light. Two aliquots of plasma were separated in a centrifuge at 2.643 g for 10 min and stored frozen at − 20 °C protected from light until their analysis (within 7 days of arrival - stability of the vitamin C molecule is 7 days at − 15 °C).

To extract and stabilize vitamin C an equal volume of cold metaphosphoric acid was added to the samples while kept on ice bath and protected from light exposure. Plasma vitamin C was analyzed by a reversed-phase high-performance liquid chromatography (HPLC) method with photodiode detector (wavelength set to 245 nm). The method was fully validated in terms of linearity (1.5–30 mg/L), imprecision (less than 5%) and accuracy [5].

The differences observed between the series by Tomasa-Irriguible et al. and ours can be explained by three reasons. First of all, the timing of the sample collection; thus we consider this the most important consideration regarding the difference in the results. While they studied vitamin C in the first 24 hours after ICU admission, our study reported vitamin C serum levels at 17.5 ± 1.7 days from admission to ICU. Second, the levels considered low by Tomasa (0.14 ± 0.05 mg / dL) would have been considered undetectable by the stoppage limit of our laboratory. So if their patients had been studied by our detection threshold, there would be 65 patients (82%) who would present undetectable levels. Third, our patients had a higher degree of lung inflammation. In our series, 94.4% of patients required prone position with P_aO_2/F_iO_2 ratio at the time of vitamin C measurement of 94.4 ± 5.9, unlike the series with which we compared

*Correspondence: ruizrodriguezjc@gmail.com

[1] Intensive Care Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain
Full list of author information is available at the end of the article

ourselves, where only 49 (73.1%) required mechanical ventilation.

We consider that our preanalytical processing of the samples for the analysis of vitamin C was carried out under conditions that guarantee the absence of oxidation of vitamin C and stability of the sample throughout the whole process. The method is robust for clinical measurement of vitamin C in plasma specimens. Consequently, the results of our study should be considered correct.

Abbreviations

ARDS: Acute Respiratory Distress Syndrome; COVID-19: Coronavirus Infectious Disease 2019; ICU: Intensive Care Unit; ml: Milliliters; PaO2/FiO2 ratio: Arterial oxygen pressure (PaO2), inspired fraction of oxygen (FIO2); SARS-CoV-2: Severe acute respiratory syndrome Coronavirus-2.

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Authors' contributions

All the authors carried out assistance and research work associated with the Vall d'Hebron Campus. ** Ruiz-Rodríguez JC MD, PhD: constructing an idea or hypothesis for manuscript, planning methodology, organising the course of the Project, taking responsibility in data management and reporting, taking responsibility in logical interpretation and presentation of the results, literature review, reviewing the article before submission for its intellectual content, writer. ** Chiscano-Camón L MD: planning methodology, organising the course of the Project, taking responsibility in data management and reporting, taking responsibility in logical interpretation and presentation of the results, literature review, writer. ** Roser Ferrer MD, PhD: literature review, reviewing the article before submission for its intellectual content, writer. ** Silvia Camos MD: literature review, reviewing the article before submission for its intellectual content, writer. ** Adolf Ruiz-Sanmartin MD: literature review, reviewing the article before submission for its intellectual content. **Ricard Ferrer MD, PhD: reviewing the article before submission for its intellectual content. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable, as we made no intervention but only analytic measurement. Moreover, the study was approved by the local Clinical Research Ethics Committee (PR(AG)270/2020) with exemption from informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Intensive Care Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain. [2]Shock, Organ Dysfunction and Resuscitation Research Group, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain. [3]Departament de Medicina, Universitat Autònoma de Barcelona, Bellaterra, Spain. [4]Biochemistry Department, Clinical Laboratories, Vall d'Hebron University Hospital, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain.

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