How important is the assessment of soluble ACE-2 in COVID-19?

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Dear editor,

We have read with interest the manuscript from Rieder and collaborators.[1] They showed that patients with COVID-19 did not exhibited changes in the renin-angiotensin-aldosterone system (RAAS). Interestingly, sACE-2 was unchanged in infected patients. However, they recognized that the activity of these markers in severe COVID-19 remains elusive. In addition, data about sACE-2 activity in recovered patients is unknown.

We conducted a cross-sectional study of 80 subjects to estimate the levels of sACE-2 in a group of severe but not critically ill confirmed COVID-19 patients by reverse transcriptase polymerase chain reaction (RT-PCR) and with less than 2 weeks from onset symptoms (N=23), a group of recovered patients (i.e., remission of symptoms or weaning of oxygen after 30 days) in whom two consecutive negative RT-PCR results from nasopharyngeal swabs within 48 hours were registered (N= 35), and a pre-pandemic control group (i.e., samples collected 4 years before the onset of the pandemic - N= 22).

sACE-2 was measured by enzyme linked immunosorbent assay (ELISA) using the commercial kit Biovision (San Francisco, CA, USA) following the manufacturer's instructions. In brief, the kit is a quantitative sandwich ELISA, with a detection range 0.391-25 ng/mL. Samples were 1:3 diluted. We made a linear model adjusted by sex and age to estimate the differences among groups. Correlation analysis was performed using the Spearman test for non-parametric data. This study was approved by the Institutional Review Board of the Universidad del Rosario. Written informed consent was solicited from each individual.

There was no significant difference in the levels of sACE-2 among groups (Figure 1A and B). Significant correlations among levels of sACE-2 and clinical parameters in severe COVID-19 patients were not observed, including viral load (Figure 1C). Network analysis revealed that sACE-2 was not close to other inflammatory markers (Figure 1D). This data suggests sACE-2 activity is unchanged in severe COVID-19 and remains unaltered in about 1 month after clinical recovery. In addition, absence of correlations advocate for a lack of value for sACE-2

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as biomarker of COVID-19 severity. This is in line with recent evidence that did not found any effect (i.e., helpful or deleterious) of RAAS inhibitors in COVID-19.[2]

In addition, there was not a correlation between sACE-2 and viral load. Recombinant sACE-2 have been recently suggested as therapeutic option for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[3] Our findings confirms that levels sACE-2 are not associated with viral load in severe patients. In conclusion, current data do not support routine use of sACE-2 in the follow-up of patients with COVID-19 and confirms that levels ACE-2 do not influence severity of disease in COVID-19.

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Ethics approval and consent to participate

All individuals singed informed consent prior participation in this study.

Competing interests

The authors declare that they have no competing interests.

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Figure 1. sACE-2 and clinical parameters in severe COVID-19. **A.** * Healthy individuals whose samples were collected prior to 2020 pandemic, avoiding the probability of SARS-CoV-2 infection. **B.** Boxplots of sACE-2 ng/mL concentrations in pre-pandemic controls, recovered patients and severe COVID-19. Not statistical differences by a linear model adjusted by age and sex were found: COVID-19 vs Pre-pandemic controls (Estimated difference (ED)= -4.251, *P*= 0.9667), COVID-19 vs recovered patients (ED= -5.049, *P*= 0.2939), and recovered patients vs. pre-pandemic controls (ED= 0.798, *P*= 1). **C.** Correlation matrix for sACE-2 and clinical parameters in COVID-19 by Spearman correlation and corrected for multiple comparison by Holm's method. sACE-2 was not correlated with any clinical parameter evaluated. Significant correlations (p value <.05) are shown with *. **D.** Network correlation analysis for sACE-2 and clinical parameters in COVID-19 by Spearman correlation. Coefficient correlations greater than |0.2| are shown. sACE-2 was not correlated with other clinical parameters and did not cluster with previously recognized factors for severity in COVID-19 such as procalcitonin, CRP, D-Dimer, ferritin, nor 4C mortality score. SD: standard deviation; COVID-19: coronavirus disease 2019; sACE-2: soluble angiotensin-converting enzyme 2; CRP: C reactive protein; SOFA: sequential organ failure assessment; BMI: body mass index.

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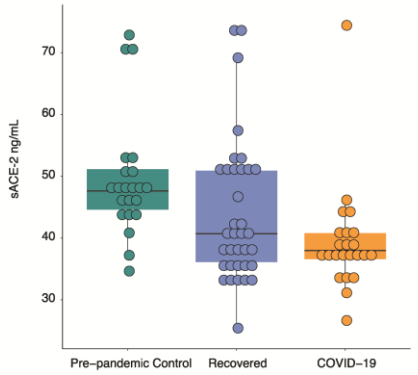
Figure 1

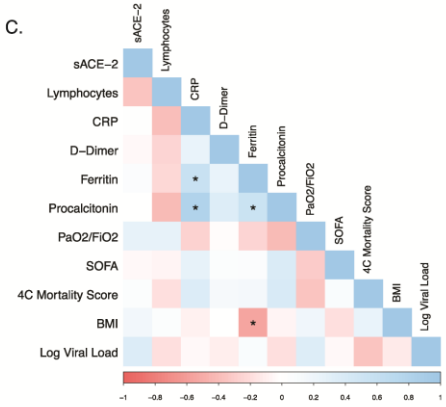
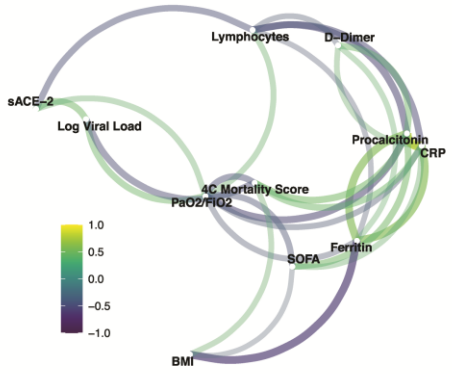
A.

Variable	Severe COVID-19 (N= 23)	Recovered (N= 35)	Pre-pandemic controls (N= 22)*
Sex (%)			
Female	8 (34.8%)	3 (8.6%)	21 (95.5%)
Male	15 (65.2%)	32 (91.4%)	1 (4.5%)
Age (Mean – SD)	49.739 (8.761)	44.829 (10.405)	29.773 (7.470)
BMI	30.126 (3.976)	NA	NA
sACE-2 (Mean - SD)	39.362 (8.816)	44.031 (11.550)	49.623 (9.916)
Days from symptoms to inclusion (Mean – SD)	7.957 (2.771)	NA	NA
Laboratories (Mean – SD)			
Lymphocytes	896.087 (364.227)	NA	NA
CRP (mg/L)	157.480 (100.952)	NA	NA
D-Dimer (mg/L)	74.461 (241.407)	NA	NA
Ferritin (ng/mL)	1917.368 (2639.320)	NA	NA
Procalcitonin (ng/mL)	0.315 (0.513)	NA	NA
PaO2/FiO2	213.486 (92.269)	NA	NA
SOFA	2.043 (0.475)	NA	NA
4C Mortality Score	6.174 (2.103)	NA	NA

B.



C.



D.