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BRIEF REPORT

Serum ACE activity and plasma ACE concentration in patients with SARS-CoV-2 infection

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ABSTRACT

Significant controversy has arisen over the role of the renin-angiotensin-aldosterone system (RAAS) in COVID-19 pathophysiology. In this prospective, observational study, we evaluated plasma angiotensin converting enzyme (ACE) concentration and serum ACE activity in 52 adults with laboratory-confirmed SARS-CoV-2 infection and 27 non-COVID-19 sick controls. No significant differences were observed in ACE activity in COVID-19 patients versus non-COVID-19 sick controls (41.1 [interquartile range (IQR): 23.0–55.2] vs. 42.9 [IQR 13.6–74.2] U/L, $p = .649$, respectively). Similarly, no differences were observed in ACE concentration in COVID-19 patients versus non-COVID-19 sick controls (108.4 [IQR: 95.8–142.2] vs. 133.8 [IQR: 100.2–173.7] μg/L, $p = .059$, respectively). Neither ACE activity ($p = .751$), nor ACE concentration ($p = .283$) was associated with COVID-19 severity. Moreover, neither ACE activity, nor ACE concentration was correlated with any inflammatory biomarkers.

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Introduction

Since angiotensin converting enzyme 2 (ACE2) was first identified as primary human host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), significant intrigue arose as to a potential pathophysiologic role of the renin-angiotensin-aldosterone system (RAAS) in coronavirus disease 2019 (COVID-19) [1]. Moreover, given that RAAS-modifying drugs (e.g. ACE inhibitors (ACEi), angiotensin receptor blockers (ARBs)) are among the most readily available and commonly prescribed medications in the world, speculation ensued over the potential therapeutic benefits and harms to such anti-hypertensive agents in COVID-19 [1].

In a recent report, Guler et al. [2] showed no significant differences in serum ACE activity between patients with COVID-19 and healthy controls, nor between those with mild compared to those with severe illness. These observations were in agreement with ours and others with respect to circulating Angiotensin II (Ang II) and aldosterone levels in COVID-19 patients, in whom no significant differences were observed between COVID-19 patients and healthy controls, as well as by COVID-19 severity [3,4]. However, we also observed significant decreases in both Angiotensin I (Ang I) and Angiotensin 1,7 (Ang 1,7) levels in patients with COVID-19 compared to healthy controls, with Ang 1,7 trending lower with increased disease severity [5]. Such findings would suggest a potential disturbance of ACE. In this report, we aimed to confirm the findings of Guler et al. [2] with respect to serum ACE activity, as well as measure plasma ACE concentration, which could also contribute to RAAS imbalance in patients with COVID-19.

Methods

Adults with symptoms suggestive of SARS-CoV-2 infection presenting to the University of Cincinnati Medical Center (UCMC) Emergency Department (ED) and with clinically indicated blood draw were prospectively enrolled *via* institutional review board-approved waiver of informed consent. Samples were centrifuged at 2,000 g for 15 min and frozen at −80 °C until analysis. Inclusion in COVID-19 cohort was dependent on positive result of reverse transcription polymerase chain reaction (RT-PCR) test for COVID-19 on standard-of-care nasopharyngeal swabs. RT-PCR negative patients were deemed non-COVID-19 sick controls, after confirmation of negative infection status using clinical criteria (CoronaScore) and serology testing, in an algorithm previously described [6,7]. Plasma concentration of ACE was measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA), with

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manufacturer's reference range between 58 and 211 μg/L. Serum ACE activity was measured using an ACE Kinetic Enzymatic Assay (Buhlmann, Amherst, New Hampshire, USA), on a Dimension RxL Max Chemistry Analyzer (Siemens AG, Munich, Germany), with manufacturer's reference range between 20 and 70 U/L.

Patients in COVID-19 cohort were stratified based on peak severity during course of infection, as having mild (ambulatory, $n = 19$), moderate (hospitalized, $n = 17$) and severe (requiring intensive care unit admission or death, $n = 16$) illness. Continuous data was reported as median and interquartile range (IQR), whilst categorical data was shown as absolute and relative frequencies. With regards to the comparison between COVID-19 positive patients and non-COVID-19 sick controls, the Mann-Whitney U test was used to identify significant differences between laboratory values, and Fisher's exact test to identify significant differences between categorical variables. Comparisons of plasma concentrations of ACE activity and concentration, along with other laboratory values, between patients with different severity levels were performed using the Kruskal Wallis test, followed by the Dunn-Bonferroni test for multiple comparisons when necessary, whilst comparisons of categorical variables were done using Fisher's exact test. The relationship between ACE activity and concentration in COVID-19 positive patients and inflammatory biomarkers was examined using Spearman's correlation coefficient. Statistical analysis was conducted using R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria), and a $p < .05$ was considered statistically significant.

Results

A total of 79 patients were enrolled, 52 with laboratory confirmed COVID-19 and 27 non-COVID-19 sick controls. Basic patient characteristics and demographics are presented in Table 1. No significant differences were found for median age ($p = .706$) and sex ($p = .219$) between groups. Eighteen (34.6%) patients in the COVID-19 cohort were taking an ACEi or ARB, while 7 (25.9%) non-COVID-19 sick controls patients were on an ACEi or ARB ($p = .611$). No significant differences were observed in ACE activity in COVID-19 patients versus non-COVID-19 sick controls (41.1 [IQR: 23.0–55.2] vs. 42.9 [IQR 13.6–74.2] U/L, $p = .649$, respectively) (Figure 1(A)). Similarly, no differences were observed in ACE concentration in COVID-19 patients versus non-COVID-19 sick controls (108.4 [IQR: 95.8–142.2] vs. 133.8 [IQR: 100.2–173.7] μg/L, $p = .059$, respectively) (Figure 1(B)).

ACE activity did not differ between patients with mild (42.9 [IQR: 23.5–54.7] U/L), moderate (41.9 [IQR: 27.7–61.4] U/L), or severe COVID-19 (31.8 [IQR: 22.9–45.6] U/L) ($p = .751$) (Figure 1(C)). Similarly, ACE concentration did not differ between patients with mild (108.6 [IQR: 101.9–136.5] μg/L), moderate (113.7 [IQR: 90.6–163.2] μg/L), or severe COVID-19 (101.2 [IQR: 81.0–127.1] μg/L) ($p = .283$) (Figure 1(D)). No significant differences were observed with respect to ACE activity in COVID-19 patients on vs. off ACEi/ARB (29.6 [IQR: 11.4–42.7] vs. 43.8 [IQR: 29.6–57.8] U/L, $p = .089$, respectively), nor in ACE concentration (131.6 [IQR: 96.1–163.4] vs. 106.9 [IQR: 96.6–129.2] μg/L, $p = .096$, respectively). Moreover, when excluding patients on ACEi/ARBS, no difference between COVID-19 patients and non-COVID-19 sick controls was observed in ACE activity (43.8 [IQR: 29.6–57.8] vs. 49.9 [IQR: 29.3–72.5] U/L, $p = .436$, respectively), nor ACE concentration (107 [IQR: 96.6–129.2] vs. 132.5 [99.7–148.7] μg/L, $p = .118$, respectively). Finally, neither ACE activity, nor ACE concentration were significantly correlated with body mass index or any inflammatory biomarker, including neutrophil count, lymphocyte count, c-reactive protein (CRP), ferritin, procalcitonin, fibrinogen, interleukins (IL)-6, 8, 10,

Table 1. Baseline patient characteristics and demographics.

Variable	COVID-19 Patients ($n = 52$)	non-COVID-19 sick controls ($n = 27$)	p-value
Age	50.5 (39.8–66)	56 (31.5–64)	.706
Sex			
Female	22 (42.3%)	7 (25.9%)	.219
Male	30 (57.7%)	20 (74.1%)	
Race			
Black	22 (42.3%)	11 (40.7%)	.002
Hispanic	18 (34.6%)	1 (3.7%)	
White	9 (17.3%)	13 (48.1%)	
Other	3 (5.8%)	2 (7.4%)	
Hypertension	26 (50.0%)	14 (51.9%)	1.000
Coronary Artery Disease	8 (15.4%)	4 (14.8%)	1.000
Heart Failure	9 (17.3%)	6 (22.2%)	.763
Hyperlipidemia	15 (28.8%)	8 (29.6%)	1.000
Diabetes	21 (40.4%)	3 (11.1%)	.009
Chronic Obstructive Pulmonary Disease	8 (15.4%)	4 (14.8%)	1.000
Chronic Kidney Disease	6 (11.5%)	6 (22.2%)	.321
Chronic Liver Disease	7 (13.5%)	5 (18.5%)	.742
Cerebrovascular Disease	7 (13.5%)	3 (11.1%)	1.000
ACEi/ARB Usage	18 (34.6%)	7 (25.9%)	.611
Angiotensin I (pg/mL)	465.2 (42.9–599.4)	722.5 (228.2–2833.6)	.012
Angiotensin II (pg/mL)	73.7 (58.7–92.0)	61.1 (51.6–124.8)	.717
Serum ACE Activity (U/L)	41.1 (23.0–55.2)	42.9 (13.6–74.2)	.649
Plasma ACE Concentration (μg/L)	108.4 (95.8–142.2)	133.8 (100.2–173.7)	.059

*Data presented as median (IQR) or n (%). ACE: angiotensin converting enzyme; ACEi: angiotensin converting enzyme inhibitor; ARB: Angiotensin Receptor Blocker.

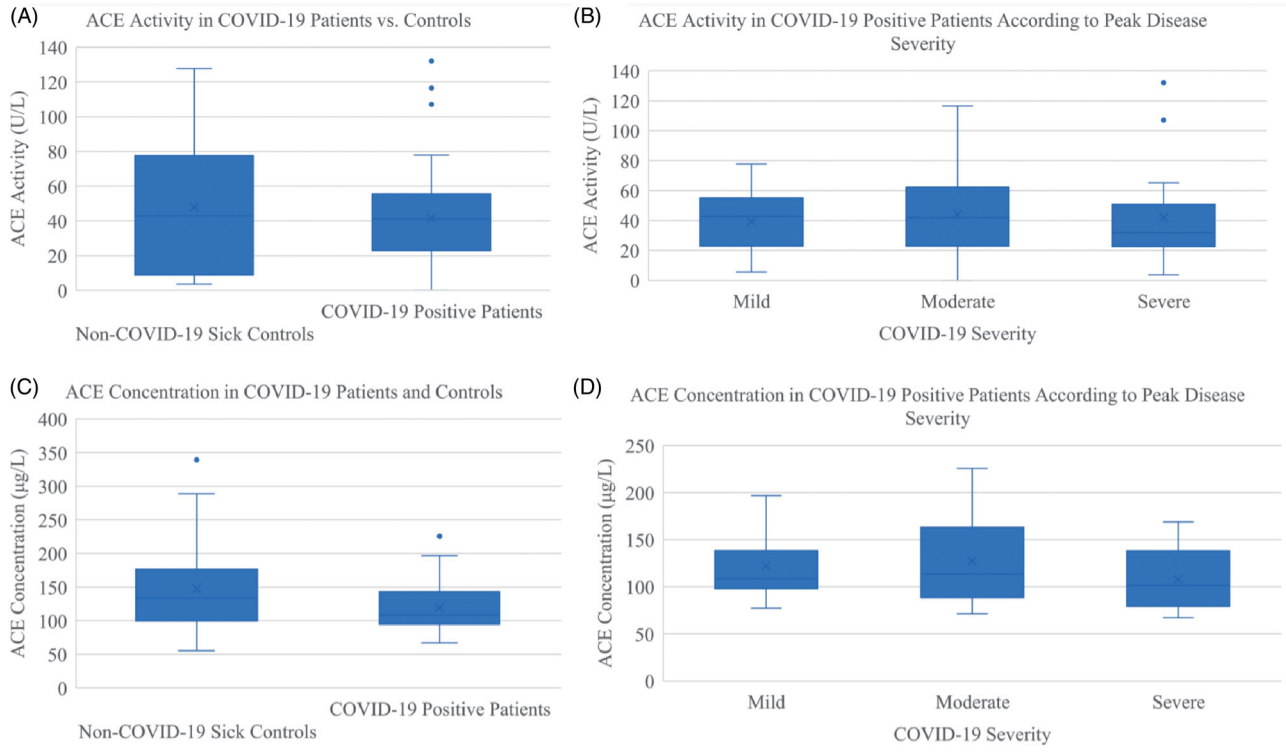

Figure 1. ACE activity and concentration in patients with and without COVID-19 (A, B) and according to COVID-19 severity (C, D).

and tumor necrosis factor-α (all $p > .05$) (Supplemental Table 1).

Discussion

In this original study, we extended earlier findings published by Guler et al. [2], confirming the lack of any significant differences in ACE activity between COVID-19 patients and non-COVID-19 sick controls. We also add that ACE concentration measured at index ED visit displays no significant differences between cases and non-COVID-19 sick controls. Moreover, we also failed to observe significant associations for either ACE parameter with respect to COVID-19 severity or inflammation.

While such observations may contribute to explain the normal Ang II values observed in our Cincinnati ED COVID-19 cohort, it does not justify previously observed low levels of Ang I which could result from alterations in ACE activity or concentration (Table 1). Moreover, we have previously reported a low Ang 1,7 state in COVID-19 [5], which has been found to be associated with other forms of ARDS [8], which could instead be explained by decreased ACE2 activity. However, the relatively normal levels of Ang II and decreased Ang I in this cohort, are not congruent with the ACE activity and level found in this study, but such findings are consistent with the abundance of literature published to-date. While an early study by Liu et al. reported to observe extremely high levels of Ang II in patients with COVID-19 [9], such findings have not been replicated in larger, well-designed investigations. In congruence with our findings in this cohort, Rieder et al. [4] observed no differences in serum concentrations of ACE 2, Ang II, or aldosterone in COVID-19 patients compared to non-COVID-19 sick controls presenting to the emergency department. Kutz et al. [10] reported a similar decrease in Angiotensin 1,7 and Angiotensin 1, as well as a decrease in angiotensin II, but no differences in ACE or ACE 2 activity, between COVID-19 patients and non-COVID-19 sick controls. Taken together, such findings suggest, at least on a systematic circulating level, a state of a depressed RAAS in COVID-19, as opposed to drastic circulating ACE2/ACE imbalance.

Overall, a more complex disturbance in the RAAS is readily apparent from measurements of major circulating parameters alone, probably requiring tissue level assessment, especially considering the tissue and organ specific regulation of ACE and ACE2 expression. Given the pre-analytic and analytic complexities in measuring angiotensin peptides, which is further exacerbated in a pandemic setting, and the need for tissue-based samples not routinely collected for clinical purposes in patients with SARS-CoV-2 infections, understanding the role of RAAS in COVID-19 continues to present a challenge.

Conclusion

In conclusion, neither ACE concentration nor ACE activity were found to be associated with COVID-19, its severity, or with the degree of inflammatory response, with values reflective of appropriate ranges at initial presentation. Further research with longitudinal measurements may hence

be needed for fully unraveling the role of RAAS aberrations in COVID-19 pathophysiology.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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