



Increased blood angiotensin converting enzyme 2 activity in critically ill COVID-19 patients

To the Editor:

Pharmacological blockade of the renin-angiotensin-aldosterone system (RAAS) with angiotensin-converting enzyme (ACE)-inhibitors and angiotensin receptor blockers (ARBs) are cornerstone treatments in several cardiovascular disease entities [1]. The RAAS is a central regulator of blood pressure, consisting of two counterregulatory pathways, commonly described as classical and nonclassical, respectively [1]. The main effect of classical RAAS activation is the generation of angiotensin (Ang)-II by ACE [1]. In contrast, non-classical RAAS activation results in cleavage of Ang-II by angiotensin converting enzyme-2 (ACE2) to form angiotensin 1-7, which directly counteracts the effects of classical RAAS activation [1].

As early as March 2020, observational studies reported associations between hypertension and coronavirus disease 2019 (COVID-19) outcome [2]. Concerns about the risk of ACE-inhibitors and ARBs were quickly raised, as these drugs were implied to upregulate ACE2, the functional receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), thereby putatively increasing SARS-CoV-2 virulence [2]. However, a recent large observational study mitigated these concerns by demonstrating neither increased susceptibility for COVID-19, nor impaired outcome of COVID-19 patients on ACE-inhibitor/ARB therapy [3]. Other observational data even suggest improved outcome of COVID-19 patients on ACE-inhibitor/ARB therapy [4], a finding currently being investigated in a randomised controlled trial (ClinicalTrials.gov identifier: NCT04311177).

Although observational studies highlight ACE2 as both friend and foe in COVID-19, there is a paucity of actual data on angiotensin metabolism in COVID-19. This is highly warranted to determine a possible causative relationship between ACE2 and COVID-19 severity.

The study was carried out in the Netherlands in accordance with the applicable rules concerning the review of research ethics committees and informed consent. All patients or legal representatives were informed about the study details and could decline to participate. The study was conducted in accordance with the declaration of Helsinki, including current revisions, and Good Clinical Practice guidelines.

We obtained blood samples from COVID-19 patients (n=10) admitted to the intensive care unit because of respiratory failure requiring invasive mechanical ventilation and from age-matched healthy controls without a history of cardiovascular disease or medication (n=5). Blood was centrifuged within 10 min following withdrawal and plasma was stored at -80°C until analysis at Attoquant Diagnostics (Vienna, Austria). To determine soluble (s)ACE2 enzyme activity, diluted plasma was spiked with Ang-II and incubated at 37°C for 1 h in the presence and absence of the specific ACE2 inhibitor MLN-4760, after which Ang-1-7 concentrations were quantified using liquid chromatography-mass spectrometry (LC-MS). The calculated difference between Ang-1-7 formation rate with/without the inhibitor results in ACE2-specific Ang-1-7-generating capacity and thus plasmatic sACE2 activity. sACE2 quantity was subsequently calculated using the linear calibration obtained from a standard curve of human recombinant



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Critically ill #COVID19 patients display markedly increased alternative angiotensin pathway activity compared to healthy controls, reflected by increased blood ACE2 levels as well as decreased angiotensin-II and enhanced angiotensin-1-7 formation <https://bit.ly/2MU1z4z>

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soluble (hrs)ACE2 in plasma obtained from healthy volunteers [5]. Ang-II levels were compared with those in non-incubated control samples to assure that the substrate was present in excess during the incubation period, assuring a stable Ang 1–7 formation rate.

Furthermore, equilibrium analysis, reflecting overall plasmatic renin–angiotensin system (RAS) activity, was performed by adding stable-isotope-labelled internal standards for angiotensin metabolites (Ang-I, Ang-II, Ang-1–7, Ang-1–5) to plasma samples followed by incubation at 37 °C for 1 h and quantification of angiotensin peptide levels by LC–MS. Data are presented as median (interquartile range) for continuous variables, and as counts and percentages for categorical variables. Between-group differences were evaluated using Mann–Whitney U-test and Fisher exact tests. A two-sided p-value <0.05 was considered significant. Data were analysed using Graphpad Prism 8.3 (Graphpad Software, San Diego, CA, USA).

Baseline characteristics were comparable between COVID-19 patients and healthy controls: age 61.5 (56.3–68.0) *versus* 58.0 (51.0–60.5) years, $p=0.22$; BMI 27.7 (25.2–30.9) *versus* 25.6 (24.3–28.1) $\text{kg}\cdot\text{m}^{-2}$, $p=0.20$; sex (male) 7/10 (70%) *versus* 4/5 (80%), $p=0.59$. Half of the patients had previously used ACE-inhibitor/ARB therapy, and 60% had a history of cardiovascular disease. All patients were critically ill and had respiratory failure, reflected by admission sequential organ failure assessment (SOFA) scores of 6 (5–8) and arterial oxygen tension (P_{aO_2})/inspiratory oxygen fraction (F_{IO_2}) ratios of 180 (111–242), respectively.

As depicted in figure 1, COVID-19 patients displayed markedly increased sACE2 levels compared with controls (13-fold increase, $p<0.001$). Moreover, the equilibrium analysis yielded increased formation of Ang-1–7 (12-fold increase, $p<0.01$; figure 1), while Ang-II formation was reduced (3-fold decrease, $p<0.01$;

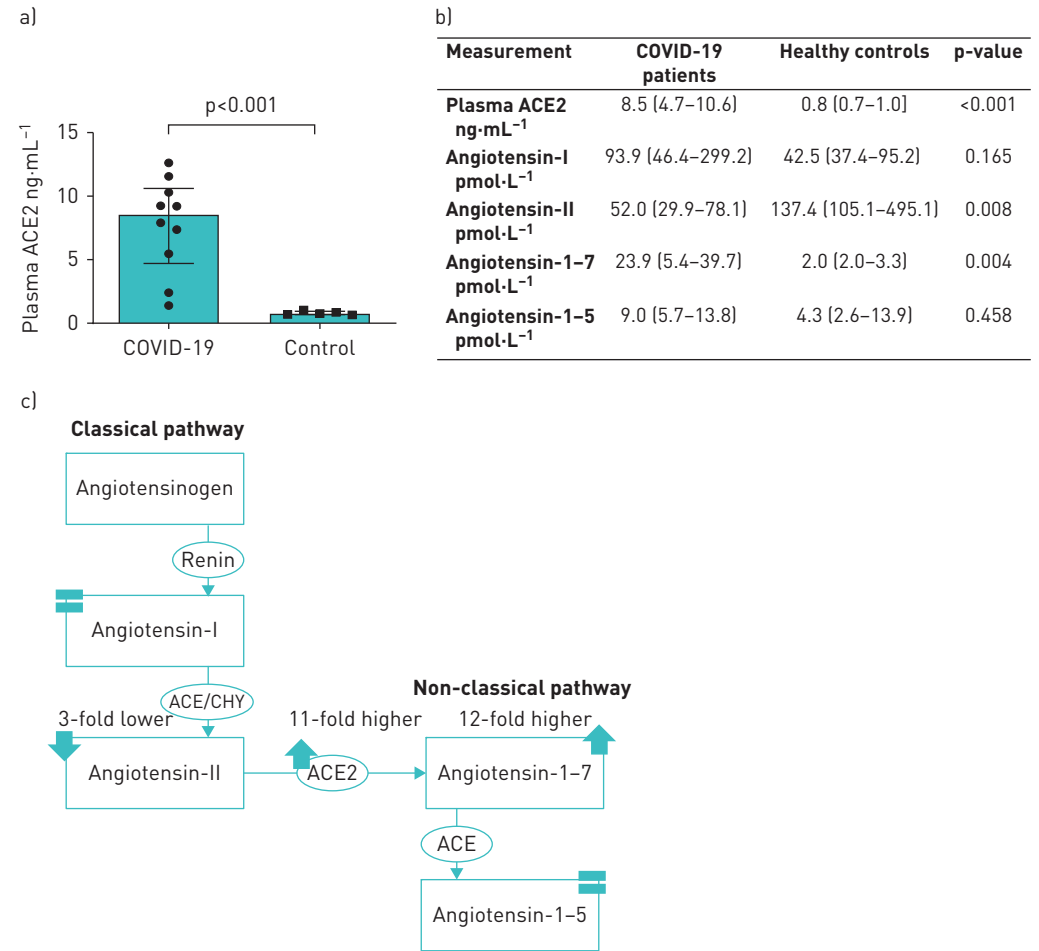


FIGURE 1 a) Plasma soluble ACE2 levels. b) Overview of all measured angiotensin enzymes/metabolites in critically ill COVID-19 patients as well as age-matched healthy controls. c) Alterations in classical/non-classical angiotensin pathways in critically ill COVID-19 patients. Median and interquartile levels are displayed, p-values were calculated using Mann–WhitneyU-tests. Arrows in panel C indicate significantly attenuated/increased levels of the respective metabolites in COVID-19 patients compared to healthy controls, while the equal sign represents a non-significant difference between groups. ACE2: angiotensin converting enzyme 2; ACE/CHY: angiotensin converting enzyme/chymase; Ang: Angiotensin.

figure 1). No differences in Ang-I and Ang-1–5 formation were observed (figure 1). Furthermore, no differences between patients previously on ACE-inhibitors/ARBs and non-users were present (data not shown).

Our data reveal increased non-classical angiotensin pathway activity in the blood of critically ill COVID-19 patients, reflected by increased sACE2 levels as well as decreased Ang-II and enhanced Ang-1–7 formation in the equilibrium assay compared with healthy controls. Whether this enhanced circulating sACE2 activity accurately reflects responses in the lung remains to be determined, as systemic angiotensin responses do not necessarily reflect local (paracrine) responses [6]. Since SARS-CoV-2 binds and cleaves membrane-bound ACE2 upon cell entry [2, 7], increased sACE2 activity might actually reflect reduced pulmonary enzyme activity, with cleaved sACE2 ending up in the circulation. Hence, our findings should be compared to measurements in the pulmonary compartment (e.g. bronchoalveolar fluid), which were unfortunately not available from our cohort.

sACE2, as well as Ang-1–7 administration was already found to protect from pulmonary injury in different murine acute respiratory distress syndrome models [8, 9]. As the ACE2/Angiotensin-1–7 axis possesses potent anti-inflammatory properties [10], the increased sACE2 levels in our cohort might also represent a failing physiological response aimed at reducing inflammation-mediated pulmonary injury. To address this, serial assessment of sACE2 activity in COVID-19 patients with varying disease severities should be performed to determine the kinetics of enhanced sACE2 activity and if it could function as a biomarker for SARS-CoV-2 disease progression.

Whether increased sACE2 levels negatively affect blood pressure in critically ill patients is currently incompletely understood. In the context of septic shock, increased classical RAAS activity is a physiological and potentially life-saving response aimed at maintaining organ perfusion [11]. For instance, it is essential to maintain glomerular filtration, especially during periods of attenuated renal perfusion [12]. As such, impaired classical RAAS activity induced by increased sACE2 levels could putatively induce hypotension in COVID-19 patients and might explain the high incidence of acute kidney injury observed in critically ill COVID-19 patients [2].

Recently, a case report described the successful treatment of a COVID-19 patient with hrsACE2 therapy [13]. A phase 2/3 study investigating hrsACE2 therapy is currently ongoing (ClinicalTrials.gov identifier: NCT04335136). Based on our data showing marked variation of circulating ACE2 levels in critically ill COVID-19 patients, sACE2 measurements may be valuable to identify patients amenable to hrsACE2 therapy in a personalised medicine approach. Along these lines, sACE2 measurements might serve as a population enrichment strategy in such trials.

A major limitation of the current study is the small sample size. Therefore, our results should be interpreted as hypothesis generating. However, it represents the first study to assess both classical and non-classical blood angiotensin metabolism in critically ill COVID-19 patients and provides initial evidence of non-classical RAAS activation in these patients.

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Data availability: The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest: D. van Lier has nothing to disclose. M. Kox has nothing to disclose. K. Santos has nothing to disclose. H. van der Hoeven has nothing to disclose. J. Pillay has nothing to disclose. P. Pickkers has nothing to disclose.

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