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RESEARCH ARTICLE

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Soluble angiotensin-converting enzyme 2 is transiently elevated in COVID-19 and correlates with specific inflammatory and endothelial markers

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Abstract

The main entry receptor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is angiotensin-converting enzyme 2 (ACE2). SARS-CoV-2 interactions with ACE2 may increase ectodomain shedding but consequences for the renin-angiotensin system and pathology in Coronavirus disease 2019 (COVID-19) remain unclear. We measured soluble ACE2 (sACE2) and sACE levels by enzyme-linked immunosorbent assay in 114 hospital-treated COVID-19 patients compared with 10 healthy controls; follow-up samples after four months were analyzed for 58 patients. Associations between sACE2 respectively sACE and risk factors for severe COVID-19, outcome, and inflammatory markers were investigated. Levels of sACE2 were higher in COVID-19 patients than in healthy controls, median 5.0 (interquartile range 2.8–11.8) ng/ml versus 1.4 (1.1–1.6) ng/ml, $p < .0001$. sACE2 was higher in men than women but was not affected by other risk factors for severe COVID-19. sACE2 decreased to 2.3 (1.6–3.9) ng/ml at follow-up, $p < .0001$, but remained higher than in healthy controls, $p = .012$. sACE was marginally lower during COVID-19 compared with at follow-up, 57 (45–70) ng/ml versus 72 (52–87) ng/ml, $p = .008$. Levels of sACE2 and sACE did not differ depending on survival or disease severity. sACE2 during COVID-19 correlated with von Willebrand factor, factor VIII and D-dimer, while sACE correlated with interleukin 6, tumor necrosis factor α, and plasminogen activator inhibitor 1. Conclusions: sACE2 was transiently elevated in COVID-19, likely due to increased shedding from infected cells. sACE2 and sACE during COVID-19 differed in correlations with markers of inflammation and endothelial dysfunction, suggesting release from different cell types and/or vascular beds.

KEYWORDS
angiotensin-converting enzyme 2, COVID-19, inflammation, renin angiotensin system, risk factor

1 | INTRODUCTION

Angiotensin-converting enzyme (ACE) 2, ACE2, is the main entry receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus causing the ongoing pandemic, with at the time of writing, more than 150 million infected and 3.2 million dead worldwide. ACE2 is a homologue of ACE and was discovered by two independent groups in 2000.[1,2] Importantly, ACE2 cleaves angiotensin II (Ang II) into angiotensin 1-7 (Ang 1-7), counteracting Ang II in the classical renin-angiotensin system (RAS).[3,4] The complete RAS system includes a number of enzymes, angiotensin peptides, and receptors, with complex interactions and feed-forward/feedback mechanisms.[3] The main RAS effector arms are considered to be ACE/Ang II/angiotensin II receptor type 1 (AT1R), mainly mediating vasoconstrictive, proinflammatory, proliferative, and pro-thrombotic effects, and ACE2/Ang 1-7/Mas receptor (MasR), which has the opposite effects,[5–7] see Figure 1. ACE2 and ACE are both membrane-anchored enzymes with wide tissue distribution.[8,9] The presence of ACE2 on different cells is thought to contribute to the varying organ manifestations seen in Coronavirus disease 2019 (COVID-19).[3,10] Both ACE2 and ACE can be shed from the cell surface in soluble forms that retain enzyme activity.[6,11] In addition to systemic RAS, which is central for cardiovascular regulation, many organs including the lungs have local RAS systems, which can partly signal independently of systemic RAS.[3,7,11]

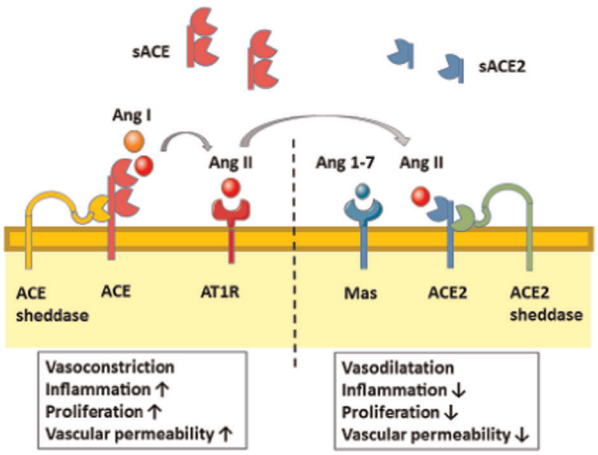

FIGURE 1 The ACE/Ang II/AT1R arm of the RAS causes vasoconstriction, is prothrombotic and increases inflammation, proliferation, and vascular permeability. ACE is shed into plasma primarily from the pulmonary microvascular endothelium by an as yet unknown sheddase. Constitutive shedding of sACE is high. The ACE2/Ang 1-7/Mas-receptor arm of RAS counteracts Ang II resulting in vasodilation and reduced inflammation, proliferation, and vascular permeability. Cell membrane ACE2 levels are primarily translationally and post-translationally regulated. Constitutive shedding of ACE2 is low and mediated primarily by the sheddase ADAM17 (a disintegrin and metalloproteinase 17). Induced shedding of ACE2 can be caused by different stimuli and may involve other sheddases. ACE2, angiotensin-converting enzyme 2; AT1R, angiotensin II receptor type 1; RAS, renin-angiotensin system

The consequences of SARS-CoV-2 interactions with ACE2 are debated, as are effects on local and systemic RAS balances and how these may contribute to COVID-19 pathology. It has been proposed that (1) high expression of ACE2 may confer increased susceptibility to SARS-CoV-2 and more severe COVID-19 and (2) that a relative lack of ACE2 in infected tissues may exacerbate local effects of Ang II, contributing to increased vasoconstriction, vascular permeability, inflammation, and thrombosis.[5,12] Experimental studies of SARS-CoV showed that virus binding, infection, and replication can downregulate cell surface ACE2 by virus-induced receptor internalization, reduced ACE2 expression, and enhanced shedding of ACE2 from the cell membrane.[13–16] Similar effects are expected for SARS-CoV-2. A lack of ACE2 can be expected to enhance lung pathology, as models of severe acute lung injury (ALI) and SARS-CoV have demonstrated a protective role for ACE2, with exacerbated lung injury in ACE2 knockouts and with ACE2 inhibition.[13,17] However, a small autopsy series of human patients who succumbed either to COVID-19 or influenza A H1N1 unexpectedly showed high ACE2 protein expression in alveolar cells and pulmonary endothelial cells for both conditions.[18] A possible explanation for this finding is interferon-induced upregulation of ACE2 which was found for humans (but not rodents) in transcriptomic studies[19,20] even if the clinical significance of this finding has been questioned.[21] Notably, many risk factors for severe COVID-19 infection such as male sex, old age, hypertension, diabetes, high body-mass index (BMI), and heart failure are associated with chronically elevated levels of soluble ACE2,[22–24] which may reflect increased ACE2 expression, enhanced ACE2 shedding or both. A pre-morbid disturbance of RAS balance could thus be a contributing factor to more severe disease in risk groups. It has also been proposed that RAS-inhibitors may increase ACE2 levels in human tissues which could either lead to increased SARS-CoV-2 susceptibility and disease severity or indeed improve resolution of ALI.[25–27]

Attention has so far focused on virus-induced changes of ACE2, but signaling in the ACE/Ang II/AT1R arm of RAS is also influenced by pulmonary disease and ALI.[11] In animal models of pneumonia, Ang II levels increased in plasma and lungs within hours of injury.[17] In human infectious ARDS, ACE activity was substantially increased in broncho-alveolar lavage (BAL) compared with healthy controls.[28] Clinical studies of ACE activity in plasma of patients with acute lung injury or ARDS showed a dynamic pattern with decrease over the

first days and, for patients who recover, subsequent normalization.[29,30] The degree and duration of the ACE decrease were shown to be associated with disease severity and has been attributed to pulmonary endothelial injury. Models of bacterial pneumonia have demonstrated the importance of a dynamic pulmonary RAS response; ACE2 in BAL and lungs initially decreased to allow entry of immune cells and then increased to limit vascular permeability and resolve inflammation.[31,32] Both RAS-effector arms may thus be involved in the pathology of COVID-19 pneumonia, and their dynamic temporal responses may influence RAS-balances locally and systemically. Shedding is expected to be a central regulating mechanism for both ACE2 and ACE. The aim of this study was to measure circulating levels of soluble ACE2 (sACE2) and ACE (sACE) during and four months after COVID-19 and to investigate their associations with the outcome and risk factors for severe COVID-19. As both sACE2 and sACE may be released from the inflamed pulmonary endothelium, we also investigated their correlations with markers of inflammation and endothelial dysfunction.

2 | PATIENTS AND METHODS

2.1 | Study population

Patients treated for COVID-19 at Danderyd Hospital April 15th to June 11th, 2020 were offered participation in the COMMUNITY project, as previously described.[33–35] Inclusion criteria were COVID-19 diagnosis, either polymerase chain reaction-confirmed (110 patients) or by clinical presentation and typical radiology (4 patients). The only exclusion criteria for the present study were age below 18 years and inability to give informed consent. The study was approved by the National Ethical Board (EPM 2020-01653). Patients were subjected to blood sampling while in hospital; plasma samples were stored in a biobank. A total of 116 patients were recruited with two patients being excluded from the present study: one was also included in an interventional study with transfusion of convalescent plasma and one lacked EDTA plasma samples at the first test. Surviving patients were offered a follow-up visit with blood sampling after four months; plasma from 58 patients was obtained in this phase. For comparison, ten healthy controls without risk factors for severe COVID-19, RAS-inhibition or cardiovascular disease (CVD) were selected from an existing biobank (local ethical board, EPN Stockholm 2015/914-31). The median age of healthy controls was 70.6 (interquartile range [IQR] 69–72) years, seven were male. All study subjects gave informed consent.

Data on medical history, status, medication, routine laboratory tests, level of care, respiratory support, and clinical development were taken from medical records. Death during hospital stay was studied as an outcome. Other indicators of disease severity were the maximum level of care (normal ward, intermediate care unit, intensive care unit) and maximum level of required respiratory support (none, oxygen <5 l/min, oxygen 5–12 l/min, high flow oxygen or noninvasive ventilation, and intubation). Analyzed risk factors for severe COVID-19 infection were age, BMI, male sex, diabetes, hypertension, known CVD, and chronic pulmonary disease. The total number of pre-existing comorbidities was individually calculated.

2.2 | Blood sampling

Blood samples were taken in resting, fasting condition in a reclining position in the morning in connection with routine blood sampling. Samples were centrifuged at 2000*g* for 20 min at room temperature within two hours of blood sampling and immediately stored at −80°C until analyses.

2.3 | Soluble ACE2 and ACE measurements

Concentrations of soluble ACE2 and ACE were measured by commercially available sandwich enzyme-linked immunosorbent assays (ELISAs): ACE2 human ELISA kit (catalog number AG-45B-0023-KI01; Adipogen Life Sciences) and human ACE ELISA kit (catalog number XPEH0026; XpressBio). Ethylenediaminetetracetic acid (EDTA) plasma aliquots were thawed on ice and analyzed in duplicate according to the manufacturer's instructions in a biosafety hood level two. For ACE2 analyses, patient plasma was diluted at 1:4 and plasma from healthy controls at 1:2. For ACE analyses all samples were diluted 1:8. Optical density (OD) was read by a Tecan platelet reader and concentrations were calculated by interpolation from the standard curve. Samples with an OD above the highest point in the standard curve were linearly extrapolated up to the maximum standard concentration plus 50%, else set to this value.

2.4 | Markers of inflammation and endothelial activation

Routine laboratory tests included C-reactive protein (CRP), blood counts, creatinine, sodium, and potassium. For subsets of patients, procalcitonin and leukocyte differential counts were available as routine tests taken at the same blood sampling occasion. Von Willebrand factor (VWF), D-dimer, factor VIII (fVIII), and plasminogen activator inhibitor 1 (PAI-1) were analyzed in another study of the COMMUNITY project, as has been reported.[33] The same study also quantified interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) using commercially available ELISAs from R&D systems (Bio-techne).

2.5 | Statistical analyses

sACE2 and sACE concentrations are presented as median with IQR as both were positively skewed. Other continuous variables are presented as mean ± standard deviation if normally distributed, otherwise as median with IQR. Categorical variables are presented as numbers and proportions. Differences between independent

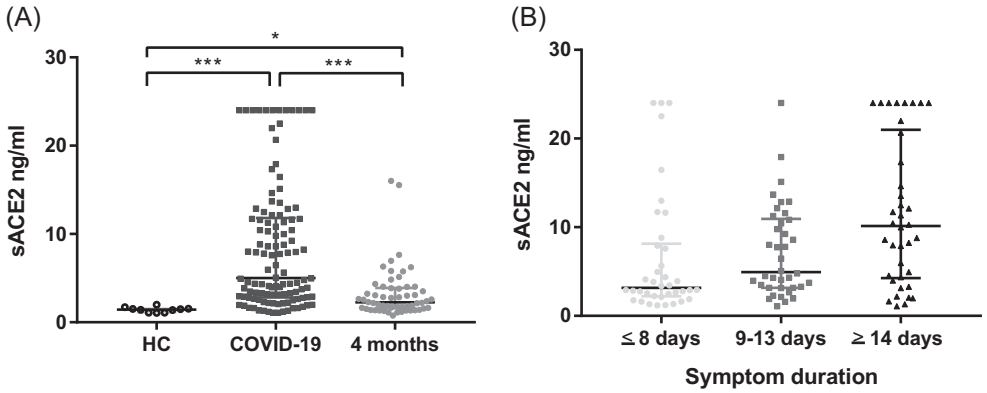

FIGURE 2 (A) Overall soluble angiotensin-converting enzyme 2 (sACE2) levels for ten healthy controls (HC), 114 COVID-19 patients during hospital admittance, and 58/114 patients sampled at a follow-up visit four months after the first test (4 months). *$p < .05$, ***$p < .0001$ by Mann–Whitney *U* test or Wilcoxon signed-rank test. (B) Acute phase sACE2 levels based on cohort tertiles depending on symptom duration at testing (38 patients per group): light grey: symptom duration ≤8 days: median sACE2 3.2 (IQR: 2.2–8.1) ng/ml, dark grey 9–13 days: sACE2 4.9 (IQR: 3.2–10.9) ng/ml, black ≥14 days: sACE2 10.1 (IQR: 4.3–21.0) ng/ml. $p = .004$ for the difference between groups by Kruskal–Wallis test. IQR, interquartile range

groups were analyzed by Mann–Whitney *U* test. Differences between sACE2 and sACE at recruitment compared to at follow-up were analyzed by Wilcoxon signed-rank test. Correlations between continuous variables were analyzed by Spearman's correlation coefficient. Differences in sACE2 and sACE depending on categorical variables were analyzed univariately by Mann–Whitney *U* test or Kruskal–Wallis test, as appropriate. *p* values <0.05 were considered significant. Statistical analyses were performed in SPSS version 26 (IBM) and Figure 2 generated by Prism (Graphpad Software Inc.).

3 | RESULTS

3.1 | Cohort characteristics

Clinical characteristics of the 114 COVID-19 patients sampled at recruitment are shown in Table 1. The majority were male (64%) and the mean age was 59 years. The most common symptoms at admittance were cough (75%), dyspnea (75%), fever (68%), diarrhea (28%), and myalgia (28%) were less common. Mean symptom duration at first blood sampling was 11.5 ± 6 days. Patients had a median of one pre-existing comorbidity (IQR: 0–2), the most common being diabetes, hypertension, and cardiovascular disease. Asthma was the most common chronic pulmonary disease. Twenty-five patients (22%) were treated at the intermediate or intensive care unit during their hospital stay. Twelve patients (10.5%) were intubated and thirteen (11.4%) patients died, twelve men and one woman.

3.2 | Soluble ACE2

Levels of sACE2 were higher in patients than healthy controls, median 5.0 (IQR: 2.8–11.8) ng/ml versus 1.4 (1.1–1.6) ng/ml, $p < .0001$, see Figure 2A. Median sACE2 levels were higher for patients with longer symptom duration at testing with the highest levels for symptom duration ≥14 days, see Figure 2B. There was also a positive correlation between sACE2 and symptom duration in days, $r = .35$, $p < .0001$. sACE2 concentrations were significantly higher for men than women, median 7.7 versus 3.8 ng/ml, $p = .021$. By univariate analysis, sACE2 levels did not differ by the presence of other risk factors for severe COVID-19, see Table 2. There was no correlation between sACE2 and the total number of comorbidities. sACE2 levels were not affected by treatment with RAS-inhibitors ($n = 37$), neither for ACE inhibitors (ACEI, $n = 19$) nor AT1R receptor blockers (ARB, $n = 18$), data not shown. There were no correlations between sACE2 and vital parameters (heart rate, respiratory frequency, blood pressure) at the time of blood sampling (data not shown). Levels of sACE2 did not differ between patients who died and survivors, and also did not depend on other indicators of COVID-19 severity (care level or required respiratory support, data not shown).

At follow-up mean four months (121 ± 14 days) after initial blood sampling, median sACE2 decreased to 2.3 (1.6–3.9) ng/ml ($p < .0001$) for the 58 patients sampled at both occasions, but remained significantly higher in patients than in healthy controls ($p = .012$), see Figure 2A. sACE2 at recruitment and sACE2 at follow-up were moderately correlated, $r = .44$, $p < .0001$. By univariate analysis, follow-up sACE2 correlated significantly with age and BMI and was higher for patients with diabetes than those without, see Table 2. Follow-up sACE2 was higher for patients with RAS-inhibitors than those without, 3.1 (2.4–4.9) versus 1.9 (1.4–3.3) ng/ml, $p = .028$ ($n = 16/58$) and correlated with the total number of comorbidities, see Table 2.

3.3 | Soluble ACE

Levels of sACE in COVID-19 patients were not significantly different from healthy controls, 57 (45–70) ng/ml versus 64 (48–265) ng/ml.

TABLE 1 Cohort clinical characteristics ($N = 114$) with markers of inflammation and endothelial dysfunction

Age, years	59 ± 15
Sex, number (%)	
Male	73 (64%)
Female	41 (36%)
BMI, kg/m^2	27.9 (IQR: 24.6–31.5)
Symptom duration, days	11.5 ± 6
Smoking	
Never	54 (47%)
Previous	28 (24.6%)
Active	3 (2.6%)
Unknown	29 (25.4%)
Diabetes, number (%)	
Type 2	26 (22,8%)
Type 1	1 (0.9%)
Hypertension, number (%)	45 (39.5%)
Cardiovascular disease, number (%)	19 (16.7%)
Congestive heart failure, number (%)	7 (6.1%)
Chronic kidney disease, number (%)	10 (8.8%)
Chronic pulmonary disease, number (%)	26 (23%)
COPD	5 (4.4%)
Asthma	18 (15.8%)
OSAS	5 (4.4%)
Cancer, number (%)	2 (1.8%)
Highest care level, number (%)	
Regular ward	89 (78%)
Intermediate care unit	10 (8.8%)
Intensive care unit	15 (13.2%)
Maximum respiratory support, number (%)	
None	39 (34.2%)
Oxygen by cannula <5 l/min	42 (36.8%)
Oxygen by cannula 5–12 l/min	16 (14%)
High flow oxygen or noninvasive ventilation	5 (4.4%)
Intubated	12 (10.5%)
CRP, mg/l ($n = 110$)	99 (62–173)
Leukocyte count, 10^9/l ($n = 109$)	6.6 (4.8–8.9)
Platelet coun, 10^9/l t ($n = 109$)	244 (172–333)
Neutrophil count, 10^9/l ($n = 97$)	4.4 (3.1–7.2)
Lymphocyte count, 10^9/l ($n = 989$	1.1 (0.8–1.5)
Monocyte count, 10^9/l ($n = 97$)	0.4 (0.25–0.6)
Procalcitonin, µg/l ($n = 67$)	0.27 (0.16–0.97)
Von Willebrand factor % ($n = 110$)	360 (248–446)
Factor VIII % ($n = 110$)	219 (161–276)
D -Dimer, mg/l ($n = 110$)	1.24 (0.73–2.50)
Interleukin 6 pg/ml ($n = 99$)	32 (14–70)
Tumor necrosis factor α, pg/ml ($n = 100$)	10 (8–21)
Plasminogen activator inhibitor 1, ng/ml ($n = 103$)	2.6 (1.7–3.6)

Note: Data are presented as mean ± standard deviation, median (interquartile range) or number (%).

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; OSAS, obstructive sleep apnea syndrome.

There was no difference in sACE levels depending on risk factors for severe COVID-19 by univariate analysis (data not shown). There was a weak correlation between sACE and total number of comorbidities, $r = .21$, $p = .023$. Levels of sACE were not affected by treatment with RAS inhibitors and did not correlate with vital parameters (data not shown). Levels of sACE did not differ depending on survival or other indicators of disease severity.

sACE at follow-up increased marginally to 72(52–87) ng/ml, $p = .008$ ($n = 58$). There was a weak to moderate correlation between sACE at recruitment and sACE at follow-up, $r = .377$, $p = .004$. Levels of follow-up sACE did not differ depending on risk factors for severe COVID-19 infection and did not correlate with the total number of comorbidities. Also, follow-up sACE did not differ depending on treatment with RAS-inhibitors.

There was no significant correlation between sACE2 and sACE during COVID-19 ($p = .788$), but a trend to a weak positive correlation between sACE2 and sACE at follow-up, $r = .25$, $p = .06$.

3.4 | Correlations of sACE2 and sACE versus markers of inflammation and endothelial dysfunction

Levels of inflammatory and endothelial markers are provided in Table 1. Significant Spearman's correlation coefficients for sACE2 and sACE versus markers of inflammation and endothelial activation with $|\text{rho}| > 0.25$ are presented in Table 3. Neither sACE2 nor sACE correlated significantly with CRP ($n = 110$), procalcitonin ($n = 67$), neutrophil count ($n = 97$) or lymphocyte count ($n = 98$). Only sACE2 had a weak positive correlation with white blood cell count. sACE2 correlated positively with monocyte and platelet counts. In contrast, acute sACE displayed negative correlations with monocyte and platelet counts. sACE2 had positive correlations with VWF, fVIII, and D-dimer, which were absent for sACE. sACE instead displayed positive correlations with IL6, TNF-α, and PAI-1 which were not seen for sACE2.

TABLE 2 Univariate analysis of acute sACE2 ($n = 114$) respectively sACE2 at follow-up ($n = 58$) after four months (sACE follow-up) versus risk factors for severe COVID-19

	sACE2		sACE2 follow-up	
	$N = 114$	p value	$N = 58$	p value
Age	$r = -0.025$	0.792, NS	$r = .288$	.029*
BMI	$r = .020$	0.838, NS	$r = .347$	.009**
Total no of comorbidities	$r = .118$	0.212, NS	$r = .399$	.002**
Male sex ($N = 73/114$; 40/58)	+102%	0.021*	NA	.181, NS
Hypertension ($N = 44/114$; 21/58)	NA	0.974, NS	+ 61%	.062
Diabetes ($N = 27/114$, 11/58)	NA	0.826, NS	+ 86%	.048*
CVD ($N = 19/114$, 9/58)	NA	0.153, NS	NA	.225, NS
Chronic pulm dis ($N = 26/114$, 11/58)	NA	0.230, NS	NA	.433, NS
Smoking ($N = 3/114$, 2/58)	NA	0.237, NS	NA	.558, NS

Note: Continuous variables: r = Spearman's correlation coefficient. Categorical variables: percentage difference in median sACE2 concentration in presence of the risk factor, p value calculated by Mann–Whitney U test. N, number of patients with presence of the risk factor.

Abbreviations: sACE2, soluble angiotensin-converting enzyme 2; BMI, body mass index, COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; NA, not applicable; NS, not significant.

4 | DISCUSSION

The main finding of this study was that levels of sACE2 were strongly elevated in hospital-treated COVID-19 patients and approached normal levels four months after infection. sACE2 levels were particularly high for symptom duration 14 days or more at testing. Men had higher sACE2 levels than women but other risk factors for severe COVID-19 did not influence sACE2 significantly. In contrast, sACE2 levels at follow-up were affected by several risk factors and treatment with RAS-inhibition and also correlated with the total number of comorbidities. sACE2 and sACE levels did not differ significantly depending on COVID-19 outcome or disease severity. sACE2 and sACE had different correlations with markers of inflammation and endothelial dysfunction, which may imply an association with different types of cell injury or release from different cell types or vascular beds.

TABLE 3 Spearman correlation coefficients for significant correlations between acute sACE2, respectively, sACE and markers of inflammation and endothelial dysfunction

	ACE2	ACE
Leukocyte count ($n = 109$)	0.26**	NS
Monocytes ($n = 97$)	0.39***	− 0.26*
Platelet count ($n = 109$)	0.32**	− 0.34**
VWF ($n = 110$)	0.36***	NS
Factor VIII ($n = 110$)	0.55***	NS
D-Dimer ($n = 110$)	0.30**	NS
IL-6 ($n = 99$)	NS	0.32**
TNF-α ($n = 100$)	NS	0.43***
PAI-1 ($n = 103$)	NS	0.44***

Note: Correlation coefficients with | rho | >0.25 are reported. N, number of patients analyzed.

Abbreviations: sACE, soluble angiotensin-converting enzyme; IL-6, interleukin 6; PAI-1, plasminogen activator inhibitor 1; TNF-α, tumor necrosis factor α; VWF, von Willebrand factor.

*$p = 0.01–0.049$.; **$p = 0.001–0.0099$.; ***$p < .001$.

To our knowledge, the sACE2 antigen has not been quantitatively measured in the acute phase of COVID-19. Two recent studies have investigated plasma ACE2 activity in COVID-19 patients, finding elevated levels.[36,37] Patel et al found high ACE2 activity one month after COVID-19; in contrast with our results, levels remained high for several months in selected patients with repeated measurements.[36] Reindl-Schwaighofer et al found ACE2 activity to be increased in hospital-treated patients with severe COVID-19.[37] Furthermore, Kragstrup et al found elevated sACE2 by semi-quantitative Olink measurements in COVID-19 patients in a recent preprint.[38] These studies combined with our results indicate that release of ACE2 to the circulation is increased in COVID-19. This may be due to increased ectodomain shedding or a combination of upregulated ACE2 protein expression in vessels/tissues[18–20] and increased shedding. Shedding of ACE2 in airway epithelial cells occurs constitutively but is also inducible.[6,14,16,39] Virus-induced shedding of ACE2 has previously been demonstrated in several studies of SARS-CoV[14–16,40] and is expected also for SARS-CoV-2. However, a number of other stimuli can increase ACE2 shedding, including cytokines,[6,16] hypoxia[6,16,41,42] and importantly high levels of Ang II.[43] These may all be present in infected tissues, potentially enhancing ACE2 shedding, which could reach the circulation due to increased vascular permeability. ADAM17 (A disintegrin and

metalloproteinase 17) is considered to be the main sheddase for ACE2 and is activated by SARS-CoV[40]; other sheddases may also contribute to inducible shedding.[16] Further research is needed to determine which cell types and shedding mechanisms contribute to the release of ACE2 into the circulation in COVID-19. Although pulmonary endothelial and/or alveolar cells are likely sources, the fact that sACE2 continues to increase late in the disease process may suggest that other cells or vascular beds contribute. sACE2 did not correlate with IL-6 or TNF-α, suggesting a limited contribution of cytokines to ACE2 shedding in COVID-19.

Downstream effects of ACE2 shedding on effector angiotensin peptides locally and systemically were not studied and require further investigation. Although an early report found high circulating ATII levels in COVID-19,[44] later studies have not reproduced this result.[45] A recent study found low levels of both angiotensin I and Ang 1–7 in COVID-19 patients.[46] High circulating levels of sACE2 may thus not overcome effects of membrane-based ACE2 with respect to circulating Ang 1–7. However, ACE2 shedding may cause a relative lack of Ang 1–7 in shedding tissues which could have pathological importance.

sACE2 levels during COVID-19 did not differ depending on the presence of risk factors for severe COVID-19 infection (with the exception of male sex) and were not affected by RAS inhibition. In contrast, sACE2 four months later correlated with age and BMI and was higher for patients with diabetes, as found by others.[22–24] Moreover, sACE2 at follow-up was higher under treatment with RAS-inhibitors and correlated with the total number of comorbidities. COVID-19-induced release of sACE2 to the circulation thus appears non-discriminate, erasing associations between sACE2 and most risk factors for severe COVID-19. Male sex deviated from this pattern, with clearly higher sACE2 levels in men than in women. The study by Patel et al and two studies of heart failure also found higher circulating sACE2 antigen/activity in men as compared to in women,[36,47,48] and also[37] found male sex to be associated with ACE2 in a mixed-effects model. This suggests that ACE2 shedding and/or expression may be higher in men under pathological conditions. It warrants further investigation if increased shedding or high sACE2 levels contribute to more severe disease in men.

In our study, sACE2 levels did not differ depending on survival or other indicators of disease severity, while associations with outcome were found in studies measuring ACE2 activity or semiquantitative antigen levels.[37,38] Groups with severe or fatal COVID-19 were small and power may have been insufficient to detect differences. However, the result may also reflect the dual nature of ACE2 in COVID-19.[5] High sACE2 levels may reflect both a high viral load and sufficient remaining tissue ACE2 to resolve inflammation. sACE2 also reduces infectivity of both SARS-CoV[16,49] and SARS-CoV-2,[50] suggesting that high sACE2 levels could limit secondary viral establishment.

Differences in sACE during COVID-19 were marginal, with slightly lower sACE during infection compared with four months later. In previous studies of ALI and ARDS, more pronounced decreases of plasma ACE activity were seen, which were attributed to injury of pulmonary microvascular endothelial cells.[29,30] The observed decrease could thus be nonspecific.

Correlations between sACE2 respectively sACE and markers of inflammation and endothelial dysfunction were investigated due to the inflammatory effect of Ang II, the anti-inflammatory effect of Ang 1–7, ACE's role in the immune system[51] and the fact that the inflamed (pulmonary) endothelium may shed both sACE2 and sACE. Correlations differed distinctly for sACE2 and sACE. sACE2 and sACE had opposite correlations with monocytes and platelets, with sACE2 being positively and sACE negatively correlated with both. Possibly shedding of ACE2 and ACE are associated with different types of endothelial activation or injury, with differences in adhesive properties, monocyte transmigration, and/or vascular permeability. Concerning platelets, recent studies have demonstrated ACE2 expression by platelets, with a possibility for SARS-CoV-2 platelet infection which could theoretically result in ACE2 shedding.[52,53] sACE2 displayed positive correlations with VWF, fVIII, and D-dimer, which were absent for sACE. VWF is thought to be released from endothelial cells in COVID-19, and the result implies that the release of sACE2 to a greater extent than sACE depends on endothelial activation. In contrast sACE correlated positively with IL-6, TNF-α, and PAI-1, which sACE2 did not. sACE is expressed in immune cells, in particular monocytes and macrophages, which may be contributing factors to sACE. It warrants further investigation if COVID-19 causes a disturbed RAS balance in the immune system and if this contributes to hyper-inflammation and pathology.

Strengths and Limitations: The study measured sACE2 antigen levels which are not influenced by the endogenous plasma ACE2 inhibitor[54] or virus-induced changes in ACE2 activity[55] and may therefore reflect shedding better than ACE2 activity. Limitations include a study of moderate size restricted to hospital-treated patients with a limited proportion of severe/fatal COVID-19. Although the cohort is considered representative, it was heterogeneous with respect to age and underlying conditions, which makes it potentially vulnerable to confounding. There are no standardized methods to measure sACE2 antigen. ELISA results were stable with median intra-assay CoV 1.8% (0.8%–3.1%) between wells and sACE2 values were similar to those of a previous study.[56] There were not sufficient spare aliquots to rerun tests at higher dilution for patients with OD above maximum concentration on the standard curve; concentrations were therefore likely underestimated for ACE2 in 16 patients and for ACE in 10 patients. The group of healthy controls could have been larger considering that levels of sACE depend on genetic polymorphisms. However, our main conclusions are based on comparisons of values during COVID-19 compared with four months later with patients being their own controls.

In conclusion, we find the plasma RAS-balance in hospital-treated Covid-19 patients to be characterized by a strong transient increase of circulating plasma sACE2 combined with a marginal reduction of sACE. Contributing factors to sACE2 elevation likely include increased shedding in infected cells, and possibly also an infection-induced increase of ACE2 membrane expression. The reduction of sACE may not be specific for COVID-19 and could be

secondary to pneumonia. We speculate that increased shedding of ACE2 may result in relative lack of Ang 1–7 in shedding tissues, but downstream effects on angiotensin peptides and also bradykinins[57] require further study. Treatment with RAS-inhibitors had no significant effect on sACE2 levels during COVID-19 and future studies on interventions targeting the RAS-imbalance may consider means to limit or compensate for ACE2 shedding, for instance by ADAM17-inhibition or substitution with Ang 1–7.

DATA AVAILABILITY STATEMENT

Data will be made available upon reasonable request due to privacy/ethical restrictions.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Concept and design: Annika Lundström, Louise Ziegler, Per Sandén; patient inclusion: Sebastian Havervall, Ann-Sofie Rudberg, Charlotte Thålin; data acquisition: Annika Lundström, Sebastian Havervall, Charlotte Thålin; laboratory analyses: Annika Lundström; analysis: Annika Lundström; interpretation: Annika Lundström, Louise Ziegler, Fien von Meijenfeldt, Ton Lisman, Nigel Mackman, Per Sandén, Charlotte Thålin; drafting of manuscript: Annika Lundström; manuscript editing: Louise Ziegler, Sebastian Havervall, Ann-Sofie Rudberg, Fien von Meijenfeldt, Ton Lisman, Nigel Mackman, Per Sandén, Charlotte Thålin

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