Serum ACE-2, angiotensin II, and aldosterone levels are unchanged in patients with COVID-19

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Abstract:

Background

The role of the renin-angiotensin-aldosterone system in COVID-19 is controversially discussed. SARS-CoV-2 enters host cells by binding to angiotensin-converting enzyme 2 and activity of the renin-angiotensin-aldosterone system may affect susceptibility to SARS-CoV-2 infection and outcome of patients with COVID-19.

Methods

In this prospective single-center study, we determined the serum levels of ACE-2, angiotensin II and aldosterone in patients with COVID-19 compared to control patients presenting with similar symptoms in the emergency unit.

Results

We analyzed serum samples from 24 SARS-CoV-2 positive and 61 SARS-CoV-2 negative patients. SARS-CoV-2 positive and control patients did not differ in baseline patients characteristics, symptoms and clinical presentation. Mean serum concentrations of ACE2, angiotensin II, and aldosterone did not differ between the SARS-CoV-2 positive and the control group. In line with this, serum potassium as surrogate parameter for RAAS activity and blood pressure were similar in both groups.

Conclusions

In summary, we did not find evidence for altered RAAS activity including angiotensin II, aldosterone, or potassium levels, and blood pressure in patients with COVID-19.

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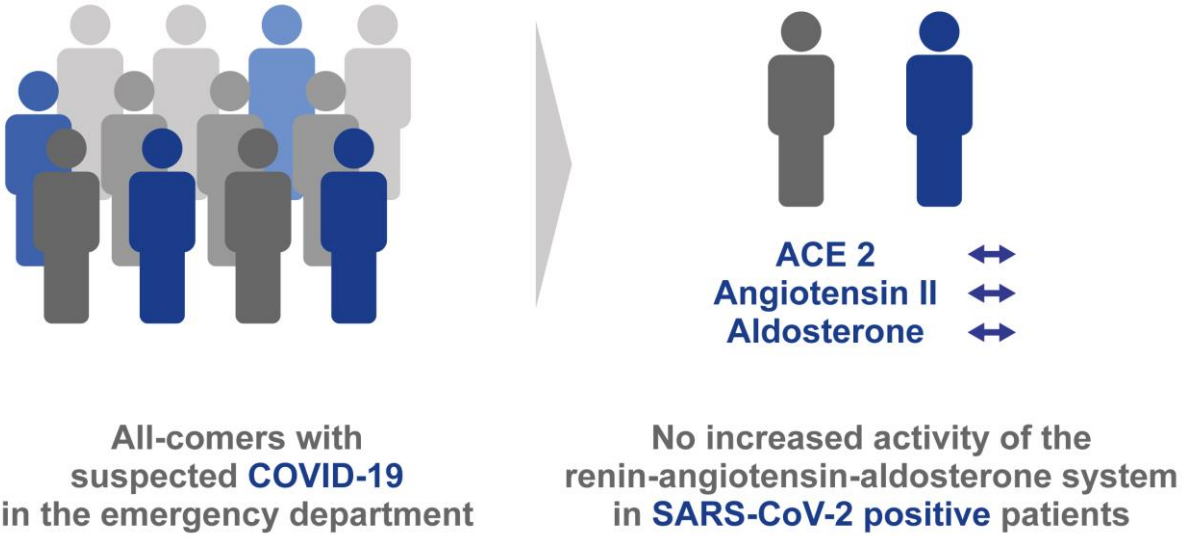
Graphical Abstract



Background: Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with significant morbidity and mortality worldwide. Since SARS-CoV-2 enters host cells by binding to angiotensin-converting enzyme 2 (ACE2)[1], the role of the renin-angiotensin-aldosterone system (RAAS) and the safety or RAAS inhibitors in COVID-19 is controversially discussed[2,3].

The formation of angiotensin II out of angiotensin I is mediated by angiotensin-converting enzyme (ACE). Angiotensin II acts via the angiotensin II receptor type 1 as a potent vasoconstrictor. In addition, it promotes the secretion of aldosterone from the adrenal gland. Both, angiotensin II and aldosterone, increase blood pressure and drive adverse tissue remodeling. Accordingly, inhibitors of the RAAS such as ACE inhibitors or angiotensin receptor blockers (ARB) are frequently prescribed drugs for hypertension and heart disease. However, the detrimental effects of ACE and angiotensin II are not limited to the cardiovascular system. Angiotensin II promotes pulmonary vasoconstriction and vascular permeability, induces the production of inflammatory cytokines and extracellular matrix synthesis, and thus contributes to many of the key characteristics of acute respiratory distress syndrome (ARDS)[4,5]. In contrast to ACE, ACE 2 mediates the breakdown of angiotensin I or angiotensin II to angiotensin-(1-9) or angiotensin-(1-7), respectively, that act as potent vasodilators[6].

Different levels of interaction between the RAAS and SARS-CoV-2 have been proposed, suggesting that ACE2 in COVID-19 may be a double-edged sword[2,5]: On the one hand, age, comorbidities, or treatment with RAAS inhibitors may alter ACE2 expression and thereby potentially increase SARS-CoV-2 susceptibility of respective patient groups[7,8]. Supplementing soluble ACE2 has been suggested as measure to block SARS-CoV-2 cell entry[9]. On the other hand, RAAS inhibition or increased plasma ACE2 expression may break down angiotensin II and attenuate its detrimental effects on ARDS in COVID-19[10]. Finally, SARS-CoV-2 infection itself may alter ACE2 expression, thus modulating angiotensin II and aldosterone levels. In this study, we determined the serum levels of ACE-2, angiotensin II

and aldosterone in patients with COVID-19 compared to control patients presenting with similar symptoms in the emergency unit.

Methods: All-comers admitted to the department of emergency medicine of the University Medical Center – University of Freiburg undergoing a PCR testing for SARS-CoV-2 from throat swab were included in this prospective single-center study (DRKS00021206, approved by the local ethical committee of the University of Freiburg (EK 153/20)). The decision to test for SARS-CoV-2 was made by the treating physician and patients were included before test results were available. We analyzed serum samples from 24 SARS-CoV-2 positive and 61 SARS-CoV-2 negative patients (control) obtained at the time point of study inclusion and determined ACE2, angiotensin II, and aldosterone by ELISA following the manufacturers' instructions (ACE2 ELISA kit, CSB-E04489h, Cusabio, Houston, TX, USA; angiotensin II ELISA kit, Enzo Life Sciences, Lausen, Switzerland; Aldosterone ELISA, IBL International GmbH, Hamburg, Germany).

Statistical analyses were performed using SPSS (version 25, IBM, SPSS Statistics, Armonk, USA) and GraphPad Prism 8 (GraphPad Software, San Diego, USA). Continuous variables were compared using student's t-test. Categorical variables were assessed by chi-square test. Correlation analysis was performed using the Spearman test for non-parametric data.

Results: SARS-CoV-2 positive and control patients did not differ in baseline patients characteristics such as age, BMI, or sex (58.3 vs 49.2 % females, p=0.479). Symptoms (rate of dyspnea, cough or fever) and clinical presentation at admission (heart rate, respiratory rate, SOFA-score) were similar in both groups, indicating comparable grades of illness. The rate of patients with previously diagnosed arterial hypertension was numerically higher in the SARS-CoV-2 negative group (33.3% for the SARS-CoV-2 positive and 50.8% for the SARS-CoV-2 negative patients), however the difference was not statistically significant (p=0.158). 33.3% of all patients from the COVID-19 group reported the intake of

ACE inhibitors, ARB, angiotensin receptor-neprilysin inhibitors, or MR antagonists, alone or in combination, compared to 36.1% in the control group (p=1.0 for overall comparison, p>0.05 for individual comparisons, Figure 1A). The use of diuretics was 20.8% in SARS-CoV-2 positive and 21.3% in SARS-CoV-2 negative patients (p=1.0). Main diagnoses in the control group were non-COVID-19 infectious diseases (56%), including 5 cases of pneumonia (8.2%), or acute cardiac disease including heart failure or coronary syndromes (14%). We performed a standardized 30-days follow-up to track the patients' clinical course. 2 participants from the SARS-CoV-2 positive cohort (8.3%) and 6 from the control group (9.8%) were admitted to ICU (p=1.0). 30-days mortality was 4.2% in the COVID-19 group and 8.2% in the SARS-CoV-2 negative cohort (p=0.671).

In our collective, angiotensin II and aldosterone levels were positively correlated with each other (Spearman r=0.3108, p=0.004). Mean serum concentrations of ACE2 (1264 pg/ml vs. 1463 pg/ml), angiotensin II (4763 pg/ml vs. 4369 pg/ml), and aldosterone (151 pg/ml vs. 185 pg/ml) did not differ between the SARS-CoV-2 positive and the control group (Figure 1 B-D). In line with this, serum potassium as surrogate parameter for RAAS activity and blood pressure were similar in both groups (Figure 1 E-G).

Conclusions: In summary, we did not find evidence for altered RAAS activity including angiotensin II, aldosterone, or potassium levels, and blood pressure in patients with COVID-19. Our study supports a very recent report describing unchanged ACE2 activity in patients with COVID-19 with or without ACE inhibitors or ARBs[11]. Using a similar approach, Kintscher and colleagues included 31 SARS-CoV-2 positive and 27 SARS-CoV-2 negative patients[11]. Though patient numbers in both studies are limited, they increase the number of reports investigating RAAS activity levels in COVID-19. While we cannot fully exclude unspecific detection of angiotensin I by the ELISA used here, Kintscher and colleagues found no difference in angiotensin II / angiotensin I peptide ratio in patients with COVID-19 as

determined by LC-MS/MS[11]. Likewise, immunoassays might overestimate absolute aldosterone levels when compared to LC-MS/MS[12], however, direct comparison of SARS-CoV-2 positive and negative patients revealed no relative change in aldosterone levels.

It is a limitation to our study, that most of the patients included had non-severe COVID-19. A smaller study from Wuhan, China, had reported that plasma levels of angiotensin II were increased in 12 cases of severe COVID-19 pneumonia compared to healthy controls and associated with disease severity[13]. More recently, it has been shown that severity of ARDS in patients with COVID-19 was associated with an increase in blood pressure and decrease in serum potassium concentrations, suggesting high RAAS activity[14]. On the other hand, we consider it a key strength of this study that we compared patients with COVID-19 to patients with other diseases but comparable characteristics and severity, not healthy individuals. This enables us to distinguish the proposed specific activation of the RAAS by SARS-CoV-2 interaction with ACE2 from unspecific activation in severe disease. Increased angiotensin concentrations have been associated with adverse outcomes of patients with ARDS[15] or heart disease[16] earlier, and our findings support the interpretation that this is not specific for COVID-19. The findings from this study are in line with a series of retrospective registries that demonstrated no effect of RAAS inhibitors on SARS-CoV-2 susceptibility or outcome of patients with COVID-19[8].

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References:

1. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Muller MA, Drosten C, Pohlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**(2)**:** 271-280 e278.

2. Hajra A, Bandyopadhyay D. COVID-19 and ACEI/ARB: Not Associated? *American journal of hypertension* 2020; **33**(8)**:** 788.

3. Schiffrin EL, Flack JM, Ito S, Muntner P, Webb RC. Hypertension and COVID-19. *American journal of hypertension* 2020; **33**(5)**:** 373-374.

4. Zhang H, Baker A. Recombinant human ACE2: acing out angiotensin II in ARDS therapy. *Crit Care* 2017; **21**(1)**:** 305.

5. Ingraham NE, Barakat AG, Reilkoff R, Bezdicek T, Schacker T, Chipman JG, Tignanelli CJ, Puskarich MA. Understanding the renin-angiotensin-aldosterone-SARS-CoV axis: a comprehensive review. *The European respiratory journal* 2020; **56**(1).

6. Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: renin-angiotensin-aldosterone system alterations. *Circulation research* 2015; **116**(6)**:** 960-975.

7. Sama IE, Ravera A, Santema BT, van Goor H, Ter Maaten JM, Cleland JGF, Rienstra M, Friedrich AW, Samani NJ, Ng LL, Dickstein K, Lang CC, Filippatos G, Anker SD, Ponikowski P, Metra M, van Veldhuisen DJ, Voors AA. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *European heart journal* 2020; **41**(19)**:** 1810-1817.

8. Verdecchia P, Cavallini C, Spanevello A, Angeli F. COVID-19: ACE2centric Infective Disease? *Hypertension* 2020; **76**(2)**:** 294-299.

9. Lutchman D. Could the smoking gun in the fight against COVID-19 be the (rh)ACE-2? *The European respiratory journal* 2020; **56**(1).

10. Felice C, Nardin C, Di Tanna GL, Grossi U, Bernardi E, Scaldaferri L, Romagnoli M, Tonon L, Cavasin P, Novello S, Scarpa R, Farnia A, De Menis E, Rigoli R, Cinetto F, Pauletto P, Agostini C, Rattazzi M. Use of RAAS inhibitors and risk of clinical deterioration in COVID-19: results from an Italian cohort of 133 hypertensives. *American journal of hypertension* 2020.

11. Kintscher U, Slagman A, Domenig O, Rohle R, Konietschke F, Poglitsch M, Mockel M. Plasma Angiotensin Peptide Profiling and ACE2-Activity in COVID-19 Patients

treated with Pharmacological Blockers of the Renin Angiotensin System. *Hypertension* 2020.

12. Guo Z, Poglitsch M, McWhinney BC, Ungerer JPJ, Ahmed AH, Gordon RD, Wolley M, Stowasser M. Aldosterone LC-MS/MS Assay-Specific Threshold Values in Screening and Confirmatory Testing for Primary Aldosteronism. *The Journal of clinical endocrinology and metabolism* 2018; **103**(11)**:** 3965-3973.

13. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Science China Life sciences* 2020; **63**(3)**:** 364-374.

14. Vicenzi M, Di Cosola R, Ruscica M, Ratti A, Rota I, Rota F, Bollati V, Aliberti S, Blasi F. The liaison between respiratory failure and high blood pressure: evidence from COVID-19 patients. *The European respiratory journal* 2020; **56**(1).

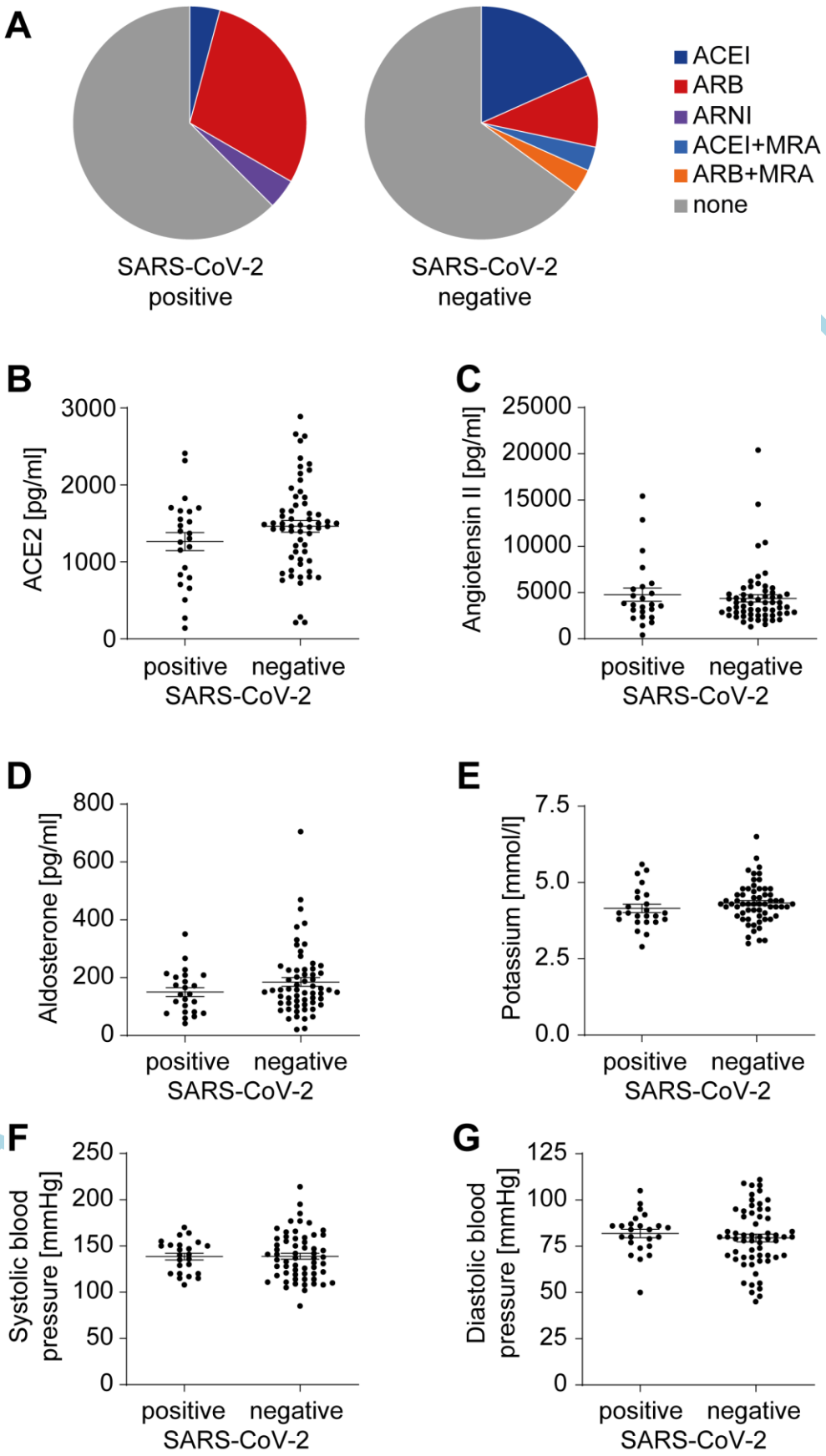
15. Reddy R, Asante I, Liu S, Parikh P, Liebler J, Borok Z, Rodgers K, Baydur A, Louie SG. Circulating angiotensin peptides levels in Acute Respiratory Distress Syndrome correlate with clinical outcomes: A pilot study. *PloS one* 2019; **14**(3)**:** e0213096.

16. Wang K, Basu R, Poglitsch M, Bakal JA, Oudit GY. Elevated Angiotensin 1-7/Angiotensin II Ratio Predicts Favorable Outcomes in Patients With Heart Failure. *Circulation Heart failure* 2020; **13**(7)**:** e006939.

Figure legends:

Figure 1: Components of the renin-angiotensin-aldosterone system in patients with COVID-19. We included SARS-CoV-2 positive and negative patients with or without ACE inhibitor (ACEI), angiotensin receptor blocker (ARB), angiotensin receptor-neprilysin inhibitor (ARNI), or mineralocorticoid receptor antagonist (MRA) treatment in a prospective study (A). Serum concentrations of angiotensin converting enzyme 2 (ACE2, B), angiotensin II (C), aldosterone (D), and potassium (E) were measured by ELISA or during routine laboratory measurement, respectively. Systolic and diastolic blood pressure (F-G) at admission was derived from medical records. Mean±SEM. SARS-CoV-2 positive (n=24) or SARS-CoV-2 negative (n=61).

Figure 1


A
ACEI
ARB
ARNI
ACEI+MRA
ARB+MRA
none
SARS-CoV-2 positive
SARS-CoV-2 negative
B
ACE2 [pg/ml]
3000
2000
1000
0
positive
negative
SARS-CoV-2
C
Angiotensin II [pg/ml]
25000
20000
15000
10000
5000
0
positive
negative
SARS-CoV-2
D
Aldosterone [pg/ml]
800
600
400
200
0
positive
negative
SARS-CoV-2
E
Potassium [mmol/l]
7.5
5.0
2.5
0.0
positive
negative
SARS-CoV-2
F
Systolic blood pressure [mmHg]
250
200
150
100
50
0
positive
negative
SARS-CoV-2
G
Diastolic blood pressure [mmHg]
125
100
75
50
25
0
positive
negative
SARS-CoV-2