


# Serum ACE2 Level is Associated With Severe SARS-CoV-2 Infection: A Cross-Sectional Observational Study

Biomarker Insights  
Volume 17: 1–6  
© The Author(s) 2022  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/11772719221125123



Amjad Bani Hani<sup>1</sup> , Nafez Abu Tarboush<sup>2</sup>, Mo'ath Bani Ali<sup>3</sup>, Fahad Alabhoul<sup>2</sup>, Fahad Alansari<sup>2</sup>, Ahmad Abuhani<sup>2</sup>, Mustafa Al-Kawak<sup>2</sup>, Badea'a Shamoun<sup>4</sup>, Suzan Albdour<sup>2</sup>, Mahmoud Abu Abeeleh<sup>1</sup> and Mamoun Ahram<sup>1</sup>

<sup>1</sup>Department of General Surgery, School of Medicine, The University of Jordan, Amman, Jordan.

<sup>2</sup>Department of Biochemistry and Physiology, School of Medicine, The University of Jordan, Amman, Jordan.

<sup>3</sup>Department of Medicine, School of Medicine, The University of Jordan, Amman, Jordan.

<sup>4</sup>Department of Anesthesia and Critical Care, Prince Hamza Hospital, Amman, Jordan.

## ABSTRACT

**OBJECTIVES:** Angiotensin-converting enzyme 2 (ACE2) represents the primary receptor for SARS-CoV-2 to enter endothelial cells, causing coronavirus disease of 2019 (COVID-19). In this study, we investigate the association between circulating ACE2 levels with the severity of COVID-19.

**METHODS:** Serum ACE2 levels were measured in 144 COVID-19-positive subjects at hospital admission, and 123 COVID-19-negative control subjects. The association between ACE2 and clinical outcomes was analyzed.

**RESULTS:** About 144 COVID-19 patients and 123 healthy controls data were analyzed, the mean age of patients was 62 years and 50% of them were males. The mean age of the control group was 55 years and 63% were males. ACE-II level was measured and compared between COVID-19 patients and controls and revealed no significant differences ( $P > .05$ ). ACE-II level was measured in COVID-19 patients and compared between different patient's subgroups, ACE II level was not dependent on gender, smoking, ACE intake, or comorbidities ( $P > .05$ ), and was significantly correlated with cardiovascular diseases (CVS) ( $P$ -value = .046) ICU admission ( $P$ -value = .0007) and Death ( $P$ -value = .0082).

**CONCLUSION:** There was no significant difference between the COVID-19 and Control group, however, ACE2 serum level was significantly higher in patients with COVID-19 who were critically ill or non-survivors, its increased level is also associated with length of stay. Elevated ACE2 level is associated with the severity of COVID-19 disease, and it has the potential to be a predictor of the severity of the disease.

**KEYWORDS:** SARS-COV2, ACE2 level, critically ill, biomarker, inflammation

**RECEIVED:** March 4, 2022. **ACCEPTED:** August 24, 2022.

**TYPE:** Original Research

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was funded by King Abdullah II Fund for Development (KAFFD), grant Number 5/2021.

**COMPETING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Amjad Bani Hani, General Surgery Department, School of Medicine, The University of Jordan, Queen Rania Street, Amman 11942, Jordan. Email: amjadbh@yahoo.com

## Introduction

In late 2019, the coronavirus disease 2019 (COVID-19) was recognized in Wuhan, China. The responsible pathogen was defined as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).<sup>1-3</sup> Since then, it has resulted in a worldwide pandemic, with more than 228 million confirmed cases and 4.6 million confirmed deaths.<sup>4</sup>

Covid-19 infection usually starts with flu like symptoms<sup>5</sup> and can be asymptomatic or may have a mild to severe course.<sup>6</sup> Admitted Patients have variable symptoms. In a study conducted in China on 1099 hospitalized COVID-19 patients, 91.1% of admitted patients developed pneumonia, 3.4% suffered from severe acute respiratory distress syndrome (ARDS) and 1.4% died.<sup>7</sup> The mechanism of viral entry into cells was identified as the angiotensin-converting enzyme 2 (ACE2), a dipeptidyl carboxypeptidase that is expressed in the human

airway epithelia, lung parenchyma, small intestine as well as in the heart, kidneys, and testes.<sup>8,9</sup>

ACE2 functions as an enzyme in the renin-angiotensin system (RAS).<sup>10</sup> The RAS plays a significant role within the physiology and pathophysiology of cardiovascular and renal systems. Renin is secreted by renal juxtaglomerular cells and converts the circulating angiotensinogen to angiotensin I (Ang I), which is then converted by the ACE1 to Ang II. The latter product harbors potent vasoconstrictive, pro-inflammatory, and pro-fibrotic properties. ACE2 cleaves Ang I into Ang (1-9), which may be converted to Ang (1-7) by ACE1. Furthermore, ACE2 degrades Ang II to Ang (1-7), which mediates vasodilatation, anti-proliferation, and antifibrosis, thereby opposing the actions of Ang II.<sup>9,11</sup> Therefore, it has been suggested that ACE2 acts in a counter-regulatory manner to ACE1 by shifting the balance between Ang II and Ang



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

(1-7), thus acting as a functional clearance mechanism for Ang II.<sup>9,11</sup>

Association between inflammatory parameters and Covid-19 infection has been studied and increased levels of inflammatory markers have been reported.<sup>12,13</sup> Moreover, patients that require multiple hospital admission have a greater inflammatory burden during the Covid-19 outbreak.<sup>14</sup> On the other hand, high ACE2 levels have been linked with an increased inflammatory burden.<sup>15</sup> Therefore, ACE2 levels could be associated with Covid-19 related inflammation.

ACE2 has been proposed as an emerging biomarker of cardiac disease.<sup>16</sup> Circulating levels of ACE2 may also have a prognostic role in monitoring COVID-19 infection.<sup>17</sup> However, the data are not yet coherent on the association between circulating ACE2 levels with the clinical and pathologic outcomes of COVID-19.<sup>18</sup> It is hoped that further studies may unravel the association of circulating ACE2 levels with the disease or its severity and promote the utilization of this information in managing COVID-19 patients. The aim of this study is to investigate the association between circulating ACE2 levels with COVID-19 infection. This is the first study in Jordan and in the middle eastern population to look at association between ACE2 level and COVID-19 infection.

## Materials and Methods

### Study subjects

One hundred forty-four (144) COVID-19 patients admitted either to Jordan University Hospital (JUH) or Prince Hamza Hospital (PHH) in Amman, Jordan, were recruited from May 23rd to June 28th, 2021. The patients were admitted to the Intensive Care Unit (ICU) or to the specialized COVID-19 hospital wards. A total of 123 healthy control subjects with no current or previous history of COVID-19 infection were recruited from Jordan University Hospital during the same period. The control group had no upper or lower respiratory tract symptoms and tested negative for COVID-19 infection. All study subjects were recruited randomly and were heterogeneous in terms of their age, gender, and the clinical presentation of patients, pediatric patients were excluded from the study. COVID-19 infection was diagnosed by a real-time, reverse-transcription polymerase chain reaction of nasopharyngeal swab samples.

The study was approved by the institutional review boards of JUH (51/2021). Safety precautions were implemented according to the Ministry of Health and Jordan University Hospital standards. Written informed consent was obtained from all healthy and infected participants.

**Inclusion and Exclusion criteria:** All patients older than 18 who were admitted to both hospitals during the recruitment period were included whereas patients younger than 18 were excluded.

### Subject data

Demographics, vital signs, comorbidities, symptoms, smoking, days of admissions, the use of ACE inhibitor drugs, and laboratory measurements were recorded for each patient following enrollment.

### ACE2 levels in serum

Five milliliters (5 mL) of venous blood were collected within the first 24 hours post-hospital admission. The serum was separated by centrifugation, aliquoted, and stored at  $-80^{\circ}\text{C}$  until used. The experimental assays were done in the Biochemistry Research Laboratories at the School of Medicine, The University of Jordan.

ACE2 was measured in 100  $\mu\text{L}$  of serum samples in duplicates using the human ACE2 DuoSet enzyme-linked immunosorbent assay plates (R&D Systems, Minneapolis, USA; DY933-05, lot: P266918) in duplicates according to the manufacturer's instructions. The concentrations of ACE2 in the samples were calculated based on a seven-point standard curve (20-0.313 ng/mL) using 2-fold serial dilutions of recombinant ACE2 provided with the kit. Biotinylated goat anti-human ACE2-antibody (842864; provided with the kit) was used to measure natural and recombinant human ACE2.<sup>19-24</sup> Considering specificity of the kit provided, the following factors prepared at 200 ng/mL were assayed and exhibited no cross-reactivity or interference (Recombinant human: ACE, Angiotensinogen, HAT, IFN- $\gamma$ , IL-4, Nephilysin, Renin, TACE/ADAM17, Angiotensin I, Angiotensin II).

### Statistical analysis

GraphPad PRISM 5 and GraphPad StatMate statistical programs were used for statistical analyses. Continuous variables are expressed as mean  $\pm$  standard error of the mean (SEM). Shapiro-Wilk normality test has been run and where appropriate, unpaired t-test or Mann Whitney test were used to compare the mean values of 2 groups, and Pearson's or Spearman's correlation coefficient analysis were used to investigating the associations of continuous variables. *P*-values of less than .05 were considered statistically significant.

## Results

The demographics of COVID-19 patients ( $n=144$ ) and controls ( $n=123$ ) are shown in Table 1. The mean age of patients was 62 years and 50% of them were males. The mean age of the control group was 55 years and 63% were males. The subjects were suffering from diabetes mellitus (54% of patients compared to 44% in control group), hypertension (50% of patients compared to 42% in control group), respiratory diseases (10% of patients compared to 7% in control group), and cardiovascular diseases (25% of patients compared to 20% in control group). 35% of COVID-19 patients were admitted to ICU. There was no difference between COVID-19 patients (23%) and controls (24%) regarding ACE drugs intake. The levels of

**Table 1.** Baseline ACE2 level, demographical, clinical characteristics of the Control group and the COVID-19 group.

PARAMETER (MEAN ± SEM) OR (%)	CONTROL	COVID-19 PATIENTS	P-VALUE
Number of Subjects/ Patients	N= 123	N= 144	
ACE2 (ng/mL)	1.9 ± 0.26	1.5 ± 0.14	.1389
Age (years)	55 ± 2	62 ± 1	.0004
Gender (M)	77 (63%)	72 (50%)	
DM	54 (44%)	78 (54%)	
HT	52 (42%)	72(50%)	
RS	9 (7%)	14 (10%)	
CVS	25 (20%)	36 (25%)	
ICU	0 (0%)	50 (35%)	
Days of admission	8.8 ± 0.9	17.2 ± 1.3	<.0001
ACE drugs	30 (24%)	33 (23%)	
Death	0 (0%)	43 (30%)	

**Table 2.** Association of ACE2 level with Age and Days of hospital admission the Control group and the COVID-19 group.

PARAMETER	CONTROL	COVID-19	
	AGE (YEARS)	AGE (YEARS)	DAYS OF ADMISSION
Number of XY pairs	123	144	144
Spearman <i>r</i>	−0.050	0.015	0.26
P-value (2-tailed)	0.5862	0.8553	0.0021

ACE2 were measured for COVID-19 patients (1.5 ng/mL) and compared to controls (1.9 ng/mL).

Table 2 shows the association of ACE-II level with age and days of hospital admission. It was revealed that ACE II level seemed to be statistically significant with “Days of hospital admission” ( $P$ -value=.0021) and was not correlated with age ( $P$ -value=.8553).

ACE-II level was measured and compared between COVID-19 patients and controls (Table 3). We tested for differences between the 2 groups and the results revealed no significant differences ( $P > .05$ ) except for respiratory and cardiovascular diseases as comorbidities ( $P < .05$ ).

ACE-II level was measured in COVID-19 patients and compared between different patients’ subgroups (Table 4). It is worth mentioning that ACE II level was not dependent on gender, smoking, ACE drugs, or comorbidities ( $P > .05$ ), and seemed to be statistically significant with Cardiovascular Diseases CVS ( $P$ -value=.0149) “ICU admission” ( $P$ -value=.0017) and “Death” ( $P$ -value=.0230).

## Discussion

COVID-19 affected individuals can be categorized into 3 main groups: (1) asymptomatic whether with or without

proven infection (2) Infected patients with mild to moderate symptoms that don’t require hospitalization (3) Moderate to Severe symptoms that require hospitalization,<sup>25,26</sup> most patients have a mild or moderate infection, but up to 5% to 10% have a severe and even life-threatening disease course with an overall mortality rate around 2% to 4%.<sup>27-29</sup>

In a Chinese study of 44 672 COVID-19 positive patients, 81% developed mild manifestations, 14% developed severe manifestations, and 5% developed critical manifestations (defined by respiratory failure, septic shock, and/or multiple organ dysfunction).<sup>30</sup> One study that included 20 133 individuals hospitalized with COVID-19 reported that 17.1% were admitted to high-dependency or intensive care units (ICUs).<sup>25,31</sup> We had 144 patients in our cohort of which 50 (35%) patients needed intensive care admission which is consistent with the experience in other groups. In a study conducted in California, USA 30% of hospitalized patients needed intensive care unit admission,<sup>32</sup> while in other reports it reaches 35%,<sup>25</sup> with a mortality rate ranging from 50% to 60% at the beginning of the pandemic to around 30% in the current time.<sup>33</sup>

Early diagnosis of the disease and the ability to predict its severity are essential for managing and preventing severe

**Table 3.** Comparison of ACE2 level between Control and COVID-19 group in association with different clinical parameters.

PARAMETER	ACE2 (NG/ML) (MEAN ± SEM)		
	CONTROL	COVID-19	P-VALUE
RS	2.4 ± 0.91	1.0 ± 0.20	.0253
CVS	1.3 ± 0.14	1.0 ± 0.15	.0194
HTN	1.8 ± 0.31	1.3 ± 0.13	.2380
DM	1.9 ± 0.24	1.6 ± 0.20	.3532
OTHER DISEASES	3.0 ± 0.95	1.9 ± 0.39	.7004
SMOKING	1.3 ± 0.17	1.0 ± 0.20	.5358
ACE DRUGS	1.7 ± 0.31	1.9 ± 0.41	.9597

morbidity and mortality. The efficacy of some of the available medications depends on the time of administration and the phase of the disease.<sup>34,35</sup> It is, therefore, imperative to find a valid novel biomarker for COVID-19. For instance, tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were tested as new predictors of COVID-19 severity.<sup>36</sup>

Although a humanized mouse model failed to detect expression in lung endothelium,<sup>37</sup> many other reports showed the expression of ACE2 in various tissues such as, esophageal epithelium, ilium and colonic enterocytes, alveolar type II cells in lung, liver cholangiocytes, myocardial cells and renal tubules.<sup>38,39</sup> These findings may indicate that clinical symptoms of organ failure or dysfunction in respiratory, renal, gastrointestinal, and cardiac system might be related to the invasion of the coronavirus in these tissues, especially in the presence of viremia.<sup>38,40</sup>

Therefore, it is believed that the ACE2 expression pattern in different tissues, could uncover the potential risk to 2019-nCoV infection because the target tissue cells expressing ACE2 might facilitate coronavirus entry.<sup>38</sup>

Endothelial cells express ACE2 receptors on their surface, which is also the primary receptor for SARS-CoV-2 where it facilitates fusion and endocytosis of SARS-CoV-2 into the pulmonary endothelial cells through the interaction of the viral spike protein with the membrane-bound ACE2.<sup>26,41</sup>

The ADAM17 (a disintegrin and metallopeptidase domain 17), which is responsible for cleaving ACE2 from the cell surface and releasing it into the circulation, can be modulated by SARS-CoV-2 spike protein.<sup>42</sup> Expression of increased shedding of ACE2 correlates with worsening of the disease, which might be the result of an increase in Ang II instead of Ang (1-7) in the first place.<sup>43,44</sup>

Bronchoalveolar lavage samples from COVID-19 patients exhibit a critical imbalance in RAAS with increased expression of ACE2, renin, and kallikrein enzymes.<sup>45</sup> Due to the cleavage

**Table 4.** Association of ACE2 level with variable clinical parameters in COVID-19 group.

PARAMETER	ACE2 (NG/ML) (MEAN ± SEM) COVID-19 PATIENTS		
	YES	NO	P-VALUE
Critically ill	2.4 ± 0.45	1.2 ± 0.18	.0017
Deaths	2.1 ± 0.34	1.3 ± 0.14	.0230
FEMALE	1.7 ± 0.20	1.3 ± 0.20	.2449
Respiratory diseases*	1.0 ± 0.20	1.6 ± 0.15	.2935
CVS diseases**	1.0 ± 0.15	1.7 ± 0.18	.0149
HTN	1.3 ± 0.13	1.7 ± 0.25	.3439
DM	1.6 ± 0.20	1.4 ± 0.20	.3628
OTHER DISEASE	1.9 ± 0.39	1.4 ± 0.14	.0640
SMOKING	1.0 ± 0.20	1.5 ± 0.15	.8911
ACE/ARB DRUGS	1.9 ± 0.41	1.4 ± 0.14	.2176

Respiratory diseases\*: Chronic obstructive pulmonary disease (COPD), Asthma, Respiratory allergies, Occupational lung diseases, Sleep apnea syndrome. Cerebrovascular System diseases\*\*: Coronary artery disease, Pulmonary embolism, Ischemic Heart Disease, Heart failure, Valvular Heart Disease, Peripheral vascular disease, Stroke. Other Diseases: Chronic kidney disease, Acute kidney injury, Liver cirrhosis, Different cancers, Rheumatological disease.

of Ang II into Ang (1-7) by ACE2,<sup>46</sup> decreased Ang II level and increased Ang (1-7) formation were found in the presence of elevated ACE2 levels in patients with severe COVID-19 compared to healthy controls.<sup>47</sup> Nonetheless, their impact on clinical outcomes is not clearly understood.

Our results did not show any difference in ACE2 levels between the COVID-19 positive group and the control group, but the Critically ill and the non-survivor patients showed significantly higher ACE2 levels.

Serum ACE2 activity was measured in 110 critically ill and 66 severely ill COVID-19 patients and was found to correlate with COVID-19 severity and predict mortality.<sup>48</sup> The ACE2 level of 306 COVID-19 positive patients and 78 COVID-19 negative patients were analyzed and high admission plasma ACE2 in COVID-19 patients was associated with increased maximal illness severity within 28 days thus the study suggests that measuring plasma ACE2 is potentially valuable in predicting COVID-19 outcomes.<sup>49</sup> Similar results were reported on COVID-19 severity and the degree of ACE2 level.<sup>47,50-52</sup>

Therefore, increased level of ACE2 could be a result of increased ACE2 shedding due to lysis of ACE2-expressing cells due to severe Lung Infection, thus the level increases with the severity of Lung involvement.<sup>36,49,53</sup> This is also consistent with the association between ACE2 level and Length of stay that we found in our cohort.

Circulating ACE2 level is usually low in normal persons<sup>54,55</sup> and increases in association with different cardiovascular disorders, such as atrial fibrillation, heart failure, hypertension, and aortic stenosis.<sup>54-56</sup> These data suggest that elevated circulating ACE2 may predispose patients to severe COVID-19 and that SARS-CoV-2 infection can further increase ACE2 levels.<sup>57</sup> In our cohort, COVID-19 positive patients had a slightly significantly higher level of ACE2 than the control group, we believe that the cardiovascular disease might have rendered the patients susceptible to infection or affected their prognosis.

The impact of age and gender was investigated on circulating ACE2 with conflicting reports, a strong relationship with age has been reported.<sup>49,58</sup> In contrast, another study found no association between age and ACE2 levels.<sup>48</sup> Male patients demonstrated higher baseline ACE2 levels, predominately in the critically ill group.<sup>48,52,59</sup> We could not demonstrate any significant association with the age or gender of either the control or the COVID-19 group. We think that the younger average age in our cohort may have contributed to our results.

#### Limitation of the study and future studies

The number of critically ill patients and the number of survivors were small. Therefore, it is difficult to generalize the findings. In addition, multiple samples taken from the same patient at different time points must also be examined to confirm the reproducibility of the findings and consistency and stability of the altered levels of circulating ACE2 in critically ill patients. In addition, ACE2 levels were studied for positive patients after COVID-19 diagnosis by PCR, so we don't know the levels of ACE2 at the beginning of symptoms before PCR.

In the future, to improve the outcomes of studies we suggest large number of COVID 19 positive patients and control group, and to enroll the positive patients with symptoms near to the date of COVID-19 swab.

#### Conclusion

There was no significant difference between the COVID-19 and Control group, however, ACE2 serum level was significantly higher in patients with COVID-19 who were critically ill or non-survivors, its increased level is also associated with length of stay. Elevated ACE2 level is associated with the severity of COVID-19 disease, and it has the potential to be a predictor of the severity of the disease. Further clinical studies investigating the association of ACE2 level with severity and course of the disease are required.

#### Declarations

##### Ethics approval and consent to participate

The study was approved by the institutional review boards of JUH (51/2021). Safety precautions were implemented according to the Ministry of Health and Jordan University Hospital standards. Written informed consent was obtained from all healthy and infected participants.

##### Consent for publication

Written informed consent for publication was obtained from all healthy and infected participants.

##### Author contributions

Study conception and design: A. Bani Hani, N. Abu Tarboush, M. Ahram. Data collection: Badea'a Shamoun, F. Alabhoul, F. Alansari, A. Abuhani, Mustafa Al-kawak. Performing the experiments: N. Abu Tarboush, S. Albdour. Analysis and interpretation of results: M. Abu Abeeleh, A. Bani Hani, N. Abu Tarboush. Draft manuscript preparation: A. Bani Hani, Mo'ath Bani Ali, M. Ahram. All authors reviewed the results and approved the final version of the manuscript

##### Acknowledgements

None.

##### Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

##### ORCID iD

Amjad Bani Hani  <https://orcid.org/0000-0003-1671-4991>

#### REFERENCES

- Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents*. 2020;55:105924.
- Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506.
- Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395:470-473.
- Tomasiuk R, Dabrowski J, Smykiewicz J, Wiacek M. Predictors of COVID-19 hospital treatment outcome. *Int J Gen Med*. 2021;14:10247-10256.
- Aktas G. A comprehensive review on rational and effective treatment strategies against an invisible enemy; SARS Cov-2 infection. *Exp Biomed Res*. 2020;3:293-311.
- Wu Z, McGoogan JM. Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323:1239-1242.
- Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020;382:1708-1720.
- Zayet S, Kadiane-Oussou NJ, Lepiller Q, et al. Clinical features of COVID-19 and influenza: a comparative study on nord Franche-Comte cluster. *Microbes Infect*. 2020;22:481-488.
- Marčetić D, Samaržija M, Vukić Dugac A, Knežević J. Angiotensin-converting enzyme 2 (Ace2) as a potential diagnostic and prognostic biomarker for chronic inflammatory lung diseases. *Genes (Basel)*. 2021;12: 1054.
- Gan R, Rosoman NP, Henshaw DJE, Noble EP, Georgius P, Sommerfeld N. COVID-19 as a viral functional ACE2 deficiency disorder with ACE2 related multi-organ disease. *Med Hypotheses*. 2020;144:110024.
- Tikellis C, Thomas MC. Angiotensin-Converting Enzyme 2 (ACE2) Is a key modulator of the renin angiotensin system in health and disease. *Int J Pept*. 2012;2012:256294.
- Aktas G. Hematological predictors of novel Coronavirus infection. *Rev Assoc Med Bras*. 2021;67:1-2.
- Khalid A, Ali Jaffar M, Khan T, et al. Hematological and biochemical parameters as diagnostic and prognostic markers in SARS-COV-2 infected patients of Pakistan: a retrospective comparative analysis. *Hematology*. 2021;26:529-542.
- Atak Tel B, Kahveci G, Bilgin S, et al. Haemoglobin and red cell distribution width levels in internal medicine patients indicate recurrent hospital admission during COVID-19. *Fam Med Primary Care Rev*. 2022;24:32-36.
- Zhang QQ, Chen FH, Wang F, Di XM, Li W, Zhang H. A novel modulator of the renin-angiotensin system, benzoylaconitine, attenuates hypertension by targeting ACE/ACE2 in enhancing vasodilation and alleviating vascular inflammation. *Front Pharmacol*. 2022;13:841435.

16. Patel SK, Velkoska E, Burrell LM. Emerging markers in cardiovascular disease: where does angiotensin-converting enzyme 2 fit in? *Clin Exp Pharmacol Physiol.* 2013;40:551-559.
17. Ciaglia E, Vecchione C, Puca AA. COVID-19 infection and circulating ACE2 levels: protective role in women and children. *Front Pediatr.* 2020;8:206.
18. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol Sex Differ.* 2020;11:29.
19. Haffke M, Freitag H, Rudolf G, et al. Endothelial dysfunction and altered endothelial biomarkers in patients with post-COVID-19 syndrome and chronic fatigue syndrome (ME/CFS). *J Transl Med.* 2022;20:138.
20. Montiel V, Lobysheva I, Gérard L, et al. Oxidative stress-induced endothelial dysfunction and decreased vascular nitric oxide in COVID-19 patients. *EBio-Medicine.* 2022;77:103893.
21. Sturrock A, Zimmerman E, Helms M, Liou TG, Paine R 3rd. Hypoxia induces expression of angiotensin-converting enzyme II in alveolar epithelial cells: implications for the pathogenesis of acute lung injury in COVID-19. *Physiol Rep.* 2021;9:e14854.
22. Elemam NM, Hasswan H, Aljaibeh H, Sulaiman N. Circulating soluble ACE2 and upstream microRNA expressions in serum of type 2 diabetes mellitus patients. *Int J Mol Sci.* 2021;22:5263.
23. Monteil V, Eaton B, Postnikova E, et al. Clinical grade ACE2 as a universal agent to block SARS-CoV-2 variants. *EMBO Mol Med.* 2022;14:e15230.
24. Reddy R, Efimenko I, Chertman W, et al. Whole exome sequencing identifies a rare mutation in NACAD as a possible cause of covid orchitis in brothers. *Urology.* 2022;159:83-86.
25. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus disease 2019 (COVID-19): a review. *JAMA.* 2020;324:782-793.
26. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* 2020;181:271-280.e8.
27. Niknam Z, Jafari A, Golchin A, et al. Potential therapeutic options for COVID-19: an update on current evidence. *Eur J Med Res.* 2022;27:1-15.
28. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, et al. Emerging treatment strategies for COVID-19 infection. *Clin Exp Med.* 2021;21:167-179.
29. Pathangey G, Fadadu PP, Hospodar AR, Abbas AE. Angiotensin-converting enzyme 2 and COVID-19: patients, comorbidities, and therapies. *Am J Physiol - Lung Cell Mol Physiol.* 2021;320:L301-L330.
30. Gao Q, Hu Y, Dai Z, Xiao F, Wang J, Wu J. The epidemiological characteristics of 2019 novel coronavirus diseases (COVID-19) in Jingmen, China. *SSRN Electron J.* 2020;2:113-122.
31. Docherty AB, Harrison EM, Green CA, et al. Features of 20133 UK patients in hospital with covid-19 using the ISARIC WHO clinical characterisation protocol: prospective observational cohort study. *BMJ.* 2020;369:m1985.
32. Myers LC, Parodi SM, Escobar GJ, Liu VX. Characteristics of hospitalized adults with COVID-19 in an integrated health care system in California. *JAMA.* 2020;323:2195-2198.
33. Auld SC, Caridi-Scheible M, Blum JM, et al. ICU and ventilator mortality among critically ill adults with Coronavirus disease 2019. *Crit Care Med.* 2020;48:e799-e804.
34. Garibaldi BT, Wang K, Robinson ML, et al. Comparison of time to clinical improvement with vs without remdesivir treatment in hospitalized patients with COVID-19. *JAMA Netw open.* 2021;4:e213071.
35. Hussain Ahsayed HA, Saheb Sharif-Askari F, Saheb Sharif-Askari N, Hussain AAS, Hamid Q, Halwani R. Early administration of remdesivir to COVID-19 patients associates with higher recovery rate and lower need for ICU admission: a retrospective cohort study. *PLoS One.* 2021;16:e0258643.
36. Henry BM, Cheruyiot I, Benoit JL, et al. Circulating levels of tissue plasminogen activator and plasminogen activator inhibitor-1 are independent predictors of Coronavirus disease 2019 severity: a prospective, observational study. *Semin Thromb Hemost.* 2021;47:451-455.
37. Muhl L, He L, Sun Y, et al. The SARS-CoV-2 receptor ACE2 is expressed in mouse pericytes but not endothelial cells: implications for COVID-19 vascular research. *Stem Cell Reports.* 2022;17:1089-1104.
38. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med.* 2020;14:185-192.
39. Zhang Z, Zhu Z, Chen W, et al. Cell membrane proteins with high N-glycosylation, high expression and multiple interaction partners are preferred by mammalian viruses as receptors. *Bioinformatics.* 2019;35:723-728.
40. Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. *Biochem Biophys Res Commun.* 2020;526:135-140.
41. Wenzel UO, Kintscher U. ACE2 and SARS-CoV-2: tissue or plasma, good or bad? *Am J Hypertens.* 2021;34:274-277.
42. Patel VB, Clarke N, Wang Z, et al. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. *J Mol Cell Cardiol.* 2014;66:167-176.
43. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res.* 2020;126:1456-1474.
44. Peron JPS, Nakaya H. Susceptibility of the elderly to SARS-CoV-2 infection: ACE-2 overexpression, shedding, and antibody-dependent enhancement (ADE). *Clinics.* 2020;75:e1912.
45. Garvin MR, Alvarez C, Miller JJ, et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. *eLife.* 2020;9:1-16.
46. Paz Ocaranza M, Riquelme JA, García L, et al. Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nat Rev Cardiol.* 2020;17:116-129.
47. van Lier D, Kox M, Santos K, van der Hoeven H, Pillay J, Pickkers P. Increased blood angiotensin converting enzyme 2 activity in critically ill COVID-19 patients. *ERJ Open Res.* 2021;7: 00848-2020.
48. Fagyas M, Fejes Z, Sütő R, et al. Circulating ACE2 activity predicts mortality and disease severity in hospitalized COVID-19 patients. *Int J Infect Dis.* 2022;115:8-16.
49. Kragstrup TW, Singh HS, Grundberg I, et al. Plasma ACE2 predicts outcome of COVID-19 in hospitalized patients. *PLoS One.* 2021;16:e0252799.
50. Patel SK, Juno JA, Lee WS, et al. Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences. *Eur Respir J.* 2021;57: 2003730.
51. Reindl-Schwaighofer R, Hödlmoser S, Eskandary F, et al. ACE2 elevation in severe COVID-19. *Am J Respir Crit Care Med.* 2021;203:1191-1196.
52. Kaur G, Yogeswaran S, Muthumalage T, Rahman I. Persistently increased systemic ACE2 activity is associated with an increased inflammatory response in smokers with COVID-19. *Front Physiol.* 2021;12:653045.
53. Filbin MR, Mehta A, Schneider AM, et al. Longitudinal proteomic analysis of severe COVID-19 reveals survival-associated signatures, tissue-specific cell death, and cell-cell interactions. *Cell Reports Med.* 2021;2:100287.
54. Úri K, Fagyas M, Kertész A, et al. Circulating ACE2 activity correlates with cardiovascular disease development. *J Renin Angiotensin Aldosterone Syst.* 2016;17:1470320316668435.
55. Fagyas M, Bánhegyi V, Úri K, et al. Changes in the SARS-CoV-2 cellular receptor ACE2 levels in cardiovascular patients: a potential biomarker for the stratification of COVID-19 patients. *Geroscience.* 2021;43:2289-2304.
56. Wallentin L, Lindbäck J, Eriksson N, et al. Angiotensin-converting enzyme 2 (ACE2) levels in relation to risk factors for COVID-19 in two large cohorts of patients with atrial fibrillation. *Eur Heart J.* 2020;41:4037-4046.
57. Montanari M, Canonico B, Nordi E, et al. Which ones, when and why should renin-angiotensin system inhibitors work against COVID-19? *Adv Biol Regul.* 2021;81:100820.
58. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med.* 2020;76:14-20.
59. Reindl-Schwaighofer R, Heinzl A, Mayrdorfer M, et al. Comparison of SARS-CoV-2 antibody response 4 weeks after homologous vs heterologous third vaccine dose in kidney transplant recipients: a randomized clinical trial. *JAMA Intern Med.* 2022;182:165-171.