Decreased serum levels of angiotensin converting enzyme (ACE)2 and enhanced cytokine levels with severity of COVID-19: normalisation upon disease recovery

Esmaeil Mortaz[1,2], Hamidreza Jamaati[3], Neda Dalil Roofchayee[1], Hakime Sheikhzade[2], Maryam Mirenayat[3], Mohsen Sadeghi[3], Somayeh Lookzadeh[3], Neda K Dezfuli[2, 4], Gert Folkerts[5], Sharon Mumby[6], Johan Garssen [5,7], Ian M. Adcock[6]

[1]Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran; [4]Department of Immunology and Laboratory Sciences, School of Allied Medical Sciences, Dezful University Of Medical Sciences, Dezful, Iran; [5]Division of Pharmacology, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands; [6]National Heart and Lung Institute, Imperial College London and the NIHR Imperial Biomedical Research Centre, London, UK;[7]Center of excellence Nutricia research,Utrecht, Netherlands;

Corresponding authors:

Prof Ian M Adcock: Ian.Adcock@imperial.ac.uk

Prof Johan Garssen: J.Garssen@uu.nl

Running title: Circulating levels of ACE2 in COVID-19

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). Circulating soluble angiotensin-converting enzyme (sACE2)2, the main receptor for SARS-CoV-2, together with components of the renin-angiotensin system promote infection and disease severity. **Objective:** In this pilot study we followed the time-course of sACE2 levels in relation to systemic cytokines in severe and moderate COVID-19 patients treated with remdesivir/dexamethasone in combination. **Methods:** Peripheral blood was obtained upon admission from 30 patients (12 with moderate disease and 18 with severe disease) and 14 patients with PCR-confirmed mild COVID-19. Severe and moderate patients were treated with remdesivir (200mg/first day and 100mg/day for the remaining days**)** and dexamethasone (100mg/day). 6 healthy control subjects (HC) were also enrolled. Serum interleukin (IL)-6 and IL-8 and sACE2 levels were measured by ELISA at baseline and during treatment in severe and moderate patients and at baseline in mild and HCs. **Results:** Baseline sACE2 levels were lower in severe (p=0.0005) and moderate (p=0.0022) patients than in patients with mild COVID-19 and in HC (p=0.0023 and p=0.0012 respectively). Serum sACE2 levels increased in patients with severe disease recovered over time compared with moderate (p=0.0021) and severe (p=0.0411) COVID-19 subjects at baseline. Systemic IL-6 and IL-8 levels were higher in all patient groups compared with HC and were not significantly affected over time or by remdesivir/dexamethasone treatment for 5 days. **Conclusion**: Serum sACE2 levels increase in severe COVID-19 patients as they recover over time whilst circulating cytokines are unaffected. Future studies should link these results to clinical outcomes.

Key words: COVID-19, remdesivir, dexamethasone, cytokines, IL-6

Introduction

Severe acute respiratory syndrome virus 2 (SARS-CoV-2) causes a respiratory disease that led to the fatal Coronavirus disease 2019 (COVID-19) pandemic (1). COVID-19 is heterogeneous and manifests clinically with fever, cough, muscle pain, fatigue, loss of taste and smell, diarrhoea and pneumonia and may result in death in susceptible subjects (2-4). Patients with poor prognostic features upon hospital admission frequently encounter complications with significant mortality associated with acute respiratory distress syndrome (ARDS), multi-organ failure and blood clots (5).

The renin–angiotensin system (RAS) maintains blood pressure and electrolyte balance in the body and has been implicated in the pathogenesis of ARDS also (6). RAS operates via two axes: the classic angiotensin converting enzyme (ACE)/Angiotensin (Ang) II/Ang II type 1 (AT_1) receptor axis and the non-classical ACE2/Ang 1–7/Mas receptor (MasR) axis. These two pathways have opposing functions: whilst the former is associated with impairment of respiratory conditions the latter plays a protective role in ARDS (7). Thus, determining the role of RAS in the pathogenesis of COVID-19 in controlling, monitoring and management of COVID-19 is essential.

ACE2 is a carboxypeptidase and type I transmembrane protein with an extracellular N-terminal domain containing the active site (8) and is recognised as a receptor for SARS-CoV-2 infection (9). ACE2 is secreted into the systemic circulation as an enzymatically active ectodomain also (8). After binding to ACE2 and entry of the SARS-CoV-2 to the target cells, shedding of host ACE2 receptors occurs that may disrupt RAS tissue homeostasis leading to important implications for COVID-19 severity (10-12). Interaction of RAS-associated proteins with circulating soluble ACE2 (sACE2) is essential for SARS-CoV-2-mediated entry (13). Furthermore, injection of recombinant ACE2 has a potential therapeutic role in treating patients infected with SARS-CoV-2 (12-14).

We hypothesised that increased sACE2 levels may protect against severe COVID-19 by blocking viral entry to target cells (15-17). Thus, we investigated the levels of sACE2 in mild, moderate and severe COVID-19 patients and assessed whether an anti-viral treatment together with dexamethasone affected sACE2 levels.

Materials and Methods

Patient sample collection

Thirty confirmed COVID-19 patients including 18 patients with severe disease, 12 with moderate disease and 14 subjects with mild disease who did not require hospitalization were enrolled into the study upon admission to the Masih Daneshvari Hospital of Shahid Beheshti Medical University (Tehran-Iran) between Jan. 10th- Feb. 5th, 2021. Six healthy control subjects (HC) were enrolled as controls. All patients were diagnosed according to World Health Organization (WHO) interim (18, 19). All COVID-19 patients were confirmed as polymerase chain reaction (PCR) positive using specific primers for SARS-CoV-2 in their nasopharyngeal samples.

Severe COVID-19 disease was confirmed by the presence of at least one of the following: respiratory rate >30/min; blood oxygen saturation ≤90% on room air; ratio of partial pressure of oxygen in arterial blood to the inspired oxygen fraction (PaO2FiO2) <300 and lung infiltrates present on >50% of the lung fields. In contrast, patients with moderate disease were characterized with respiratory rate ≥24 and SpO2 ≤93% on room air and mild disease was characterized by upper respiratory tract symptoms without shortness of breath or hypoxia (20). The study was approved by the institutional ethics board of the Masih Daneshvari Hospital (Ethics number SBMU.NRITLD.REC.1399.226).

Data collection

The clinical records of patients were interpreted by the research team of the Department of Critical Care Medicine, Masih Daneshvari Hospital of Shahid Beheshti University. Clinical, laboratory, and radiological properties and treatments and outcomes data were collated from electronic medical records. The information recorded included demographic data, medical history, underlying comorbidities, symptoms, signs, laboratory findings; chest computed tomographic (CT) scans, and treatment measures. The antiviral treatment in this study for the moderate and severe patients was remdesivir (200mg/ first day and 100mg/day for the 4 days) with dexamethasone (100mg/day) as previously described (21). Besides in all severe patients were treated with methylprednisolone (125 mg-500 mg) for three days and for rest with

dexamethasone (8-16 mg for 5 days) and the moderate patient only got dexamethasone as the same as severe patients. In addition, all patients with severe disease received a single dose of Actemra (anti-IL-6, 8 mg/kg) upon admission to hospital as recommended (22).

Laboratory examination of blood samples

Blood (3ml in tubes without anticoagulants) was obtained at baseline and 5 days after initiation of antiviral treatment and other supportive therapy. The erythrocyte sedimentation rate (ESR) was determined in citrate-treated whole blood samples. For total blood samples containing, anti-coagulant EDTA (3ml) were obtained from all participants upon admission and 7 days after antiviral therapy.

Serum samples were separated by centrifugation at 1200 x g for 5 min. Serum biochemical tests (including kidney and liver function tests including phoshocreatine kinase (CPK), lactate dehydrogenase (LDH), electrolytes, C reactive protein (CRP) and myocardial enzymes were obtained.

Analysis of serum sACE2, IL-6 and IL-8

Serum sACE2 was measured using an ELISA kit (Mybiosource, San Diego, CA) according to the following Manufactures' instructions. The human ACE2 ELISA Kit has a detection range of 62.5-4000 pg/ml. Circulating levels of IL-6 (a detection range of 9.4 – 600 pg/ml) (R&D Biosystem) and IL-8 (OptEIA™ assay, detection ≥2pg/mL)(BD Bioscience) were evaluated in the serum of participants before and after treatment according to the following manufacturer's instructions.

Statistical analysis

Analysis of data was performed using the SPSS program version 16.0 (SPSS, Inc. Chicago, USA) and GraphPad Prism software (version 8 Graph Pad Software, Inc.). A non-parametric Mann-Whitney U test (Median, 5-95% percentile was used for the variables without normal distribution. Unpaired and paired t-student tests (Mean, 95% confidence intervals (CI) were used for parametric variables. $p<0.05$ was considered as statistically significant.

Results

Demographic and clinical characteristics of patients and healthy control

Basic demographic and clinical characteristics of the participants are shown in **Table 1**. The mean age of the COVID-19 patients was not significantly different from that of the healthy controls (57.1 ± 2.6 versus 46.5 ± 6.2 years, p=0.1043) but gender was different due to all control subjects being male. Serum levels of ESR (p=0.0013), CRP (p=0.0067), LDH (p=0.0004) and CPK (p=0.0077) were higher in severe COVID-19 patients at baseline than in mild patients whilst baseline levels of all but CPK (p=0.1193) were also higher in moderate patients compared with mild patients (**Table 1**). These parameters were not measure for healthy subjects. **Table 1** also shows the respiratory rate (RR), CT values and SpO2 and APACHEII of subjects at baseline.

Over time, the serum levels of ESR (p=0.0125) and CRP (p=0.015) were significantly reduced in severe patients and as were CRP levels (p=<0.0001) in patients with moderate disease (**Table 1**). This coincided with the introduction of remdesivir and dexamethasone therapy for 5-7 days. In contrast, LDH levels were significantly elevated over time in patients with severe disease (p=0.0197) and unaltered in patients with mild disease (**Table 1**). Overall, the serum levels of ESR and CRP were similar in severe patients after 5-7 days, which included treatment with remdesivir and dexamethasone, to those seen in mild patients whilst the level of LDH (p=0.0031) and CPK (p=0.0065) remained significantly different from those in mild subjects (**Table 1**). In comparison, only the serum levels of LDH in moderate COVID-19 patients after 5-7 days were significantly different (p<0.0001) from the levels seen in subjects with mild disease (**Table 1**).

sACE2 levels at baseline and over time

Baseline sACE2 levels in severe patients were significantly lower than observed in patients with mild COVID-19 (p=0.0005) or HC (p=0.0022) (**Fig. 1A, Table 2**). Similarly, baseline sACE2 levels in moderate COVID-19 patients were also significantly lower than observed in patients with mild disease (p=0.0023) or HC (p=0.0012) (**Fig. 1B, Table 3**). No difference in baseline sACE2 level was observed between HC and mild COVID-19 patients (**Fig. 1, Table 3**). Serum levels of sACE2 were significantly up-

regulated over 5-7 days in the severe patients compared with baseline (P=0.0411) (**Fig. 1A, Table 3**). A similar up-regulation was also seen in moderate COVID-19 patients (P=0.0021) (**Fig. 1B, Table 3**). Induction of sACE2 over 5-7 days in severe and moderate COVID-19 patients resulted in similar sACE2 levels to those seen in baseline levels in subjects with mild COVID-19 and HC (**Fig. 1A & B, Table 3**).

Serum cytokines levels at baseline and over time

As a marker of the cytokine storm, serum levels of IL-6 and IL-8 were evaluated in the COVID-19 groups and HC at baseline and in the moderate and severe COVID-19 patients over 5-7 days of treatment with remdesivir and dexamethasone. Baseline levels of serum IL-6 were elevated in severe patients compared with HC (P=0.036) and were not significantly different from levels seen in mild patients (P=0.7649). Severe COVID-19 patients on remdesivir did not show a significant change in serum IL-6 levels over time (P=0.3753) (**Fig. 2A, Table 3**).

In addition, baseline serum IL-6 levels were elevated in patients with moderate COVID-19 compared to HC (P= 0.008) and similar to those observed in patients with mild disease at baseline (**Fig. 2B, Table 3**). As with severe patients, the serum IL-6 levels did not significantly alter over 5-7 days in the presence of remdesivir and dexamethasone therapy (P>0.99) (**Fig. 2B, Table 3**).

Serum IL-8 levels were significantly elevated in severe patients at baseline and remained so after 5-7 days compared with healthy subjects (P=0.008 and P=0.035, respectively). However, baseline levels of serum IL-8 in patients with severe disease were similar to levels seen in mild patents (P=0.5002). Serum IL-8 levels in severe COVID-19 patients was not affected over time concomitant with remdesivir treatment (P=0.069) (**Fig. 3A, Table 2**).

Baseline serum IL-8 levels were also raised in patients with moderate COVID-19 compared to HC (P=0.0047) and similar to those observed in patients with mild disease at baseline (**Fig. 3B, Table 2**). As with severe patients, serum IL-8 levels did not significantly change over the 5-7 days of recovery during remdesivir and dexamethasone therapy (P=0.7330) (**Fig. 3B, Table 3**).

Discussion

We hypothesized that higher serum ACE2 levels in serum of mild, moderate and severe COVID-19 patients could account for the differences of COVID-19 disease manifestation and severity. We demonstrated that sACE2 levels are lower in severe and moderate COVID-19 patients compared to mild subjects at admission. Over 5-7 days as patients recovered, sACE2 levels were enhanced in both moderate and severe patients. In contrast, although serum cytokines were elevated in all COVID-19 subjects, the levels were unchanged over the following 5-7 days. The levels of LDH in the severe COVID-19 patients increased over the duration of hospitalization which may reflect ongoing cell death in response to the host defence to infection.

These data highlight the dissociation between sACE2 and serum cytokine levels at baseline and during recovery over time.

Early diagnosis and suitable clinical monitoring and management of COVID-19 patients is critical in preventing severe consequences and even death in hospitalised COVID-19 patients (23). Multiple efforts are underway globally to inhibit virus infection to control SARS-COV-2 infection and the COVID-19 pandemic. Until global vaccination programmes achieve suitable levels, additional approaches such as blocking viral entry using transmembrane protease serine 2 inhibitors; sACE2 or antibodies, and viral RNA-dependent RNA polymerase (eg, remdesivir) may be useful (23).

ACE2 receptors plays an important role in the binding of SARS-CoV-2 and is located on the surface of many cells including respiratory epithelial cells. ACE2 receptors enables viral entry to target cells and vaccines specifically targeting the viral spike protein–ACE2 interaction are being used (24).

Therefore, ACE2 is at the core of COVID-19 research and drug development. We report here that sACE2 levels were lower in moderate and severe patients compared with those in healthy subjects and patients with mild disease and that levels are elevated over time as patients recover either as a natural response or as a result of intervention with remdesivir and dexamethasone therapy. This raises the question regarding clinical relevance of low sACE2 in moderate and severe patients and the restoration of ‘normal’ levels with time during recovery. This may reflect shedding of ACE2 following binding by SARS-CoV-2. Haga and colleagues demonstrated that binding of recombinant and

virion-associated SARS Spike (S) protein to ACE2 receptors induces ACE2 shedding in a disintegrin and metalloproteinase domain 17 (ADAM17)/tumor necrosis factor alpha (TNF-α)-converting enzyme (TACE)-dependent manner (24,25). Moreover, ACE2 shedding might reduce ACE2 surface receptor expression. Exposure of HEK293 cells to the phorbol ester phorbol myristate acetate (PMA) *in vitro* induces ACE2 shedding and reduced the amount of cell-associated ACE2 receptors (25, 26). Furthermore, incubation of cells with control virus-like particles (VLPs) which do not contain any viral glycoprotein does not induce ACE2 shedding whereas VLPs bearing the SARS-S protein triggers ACE2 shedding. Thus, SARS-CoV-2 can downregulate its receptor and the S protein might be critical to receptor interference (27).

No significant difference in serum levels of sACE2 between individuals who were PCR-positive or -negative for SARS-CoV-2 infection (28). This study, however, did not study serum levels of sACE2 in patients with differing severity of disease nor the effect of antiviral and/or dexamethasone treatment *per se* since we did not have an untreated control group due to ethical reasons.

Injection of exogenous human recombinant soluble (hrs)ACE2 in COVID-19 patients has beneficial effects (8, 17, 29, 30). Interestingly, 9 days after the onset of symptoms, intravenous infusion with hrsACE2 (APN01; 0·4 mg/kg) was successful in treating patients (30). Importantly, hrsACE2 treatment did not interfere with the generation of neutralising antibodies. The authors suggested that hrsACE2 could block the systemic spread of the virus from the lung to other organs (30).

Moreover, there is an age- and sex-relationship between ACE2 in humans. sACE2 levels are low in both sexes up to the age of 12 whereupon it increases to a greater extent in males such that males older than 15 have higher sACE2 levels than women (31). Higher levels of sACE2 are associated with a greater risk for severe COVID-19 (32) which may explain the greater susceptibility to infection by SARS-CoV-2. In addition, smoking and individuals with Type 2 diabetes and/or obesity also have higher serum sACE2 levels (33). However, plasma concentrations of sACE2 are much lower in diabetics with chronic disease than in healthy controls and are further reduced by the use of hypoglycemia drugs. This may provide a rationale for why diabetics with lower plasma levels of sACE2 may be more susceptible to severe COVID-19 (34). Having

only males in the healthy control group is a limitation of the study as is the collection of samples from only subsets of recruited patients rather than all subjects.

Serum levels of circulating sACE2 should be considered as a potential interface between chronic inflammation, cardiovascular disease and COVID-19 susceptibility (35). Elderly patients with aortic stenosis have markedly elevated serum ACE2 levels together with altered left and right ventricular functions, which may pose higher risks during COVID-19 (35). In addition, high plasma sACE2 levels may be a predictor of COVID-19 severity and outcomes as well as a risk factor for hypertension, pre-existing heart disease and pre-existing kidney disease (36). In contrast, in lung diseases circulating sACE2 levels were significantly reduced in COPD and pulmonary fibrosis (PF) compared to healthy control (37).

Thus, age and co-morbidities may be important confounders, but it is unlikely that the age of the patients is a confounding factor in this study since all of participants are adults of a similar age. Furthermore, there was no link between sACE2 levels and any underlying diabetes, smoking and hypertension in severe and moderate COVID-19 patients in this study (data not shown). Despite the changes observed in sACE2 levels we did not find any significant differences in serum IL-6 or IL-8 levels over the duration of the study. This was surprising and may reflect the timing and/or dose of the dexamethasone treatment in these patients or alternatively that levels may have risen in untreated subjects. Although there are several strengths to our study we recognize that there are some limitations. These include the low number of participants. In addition, we do not have untreated, severe COVID-19 subjects to act as controls as this would not be allowed by our Ethics Committee. Future studies are required in a larger multi-centre cohort to validate these results.

Whether the increased levels of sACE2 in the serum are a direct result of the antiviral and dexamethasone therapy or results from a normal compensatory mechanism over time to control the virus remains unknown. Interactions of SARS-CoV-2 with ACE2 shedding over time may account for the higher levels of ACE2 in mild patients than in patients with moderate and severe disease. Whether this is due to remdesivir and dexamethasone therapy is unclear. Higher levels of ACE2 over time or possibly following treatment may reflect an action of these drugs or the natural recovery from

disease that is independent of any anti-inflammatory effect and highlights the potential for direct targeting of ACE2 in treating severe COVID-19.

Figures legends

Figure 1. Serum levels of soluble angiotensin converting enzyme (ACE)2 in COVID-19 patients. (A) Dot blots of individual values and median (5-95% percentiles) of serum sACE2 levels before and after 5-7 days including remdisivir and dexamethasone therapy in severe COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. (**B**) Dot blots of individual values and median (5-95% percentiles) of serum sACE2 levels before and after 5-7 days including remdisivir and dexamethasone therapy in moderate COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. *p<0.05, **p<0.01, ***p<0.001, ns; nonsignificant.

Figure 2: Serum interleukin (IL)-6 levels in COVID-19 patients. (A) Dot blots of individual values and median (5-95% percentiles) of serum IL-6 levels before and after 5-7 days including remdisivir and dexamethasone therapy in severe COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. (**B**) Dot blots of individual values and median (5-95% percentiles) of serum IL-6 levels before and after 5-7 days including remdisivir and dexamethasone therapy in moderate COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns; non-significant

Figure 3. Serum interleukin (IL)-8 levels in COVID-19 patients. (A) Dot blots of individual values and median (5-95% percentiles) of serum IL-6 levels before and after 5-7 days including remdisivir and dexamethasone therapy in severe COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. (**B**) Dot blots of individual values and median (5-95% percentiles) of serum IL-6 levels before and after 5-7 days including remdisivir and dexamethasone therapy in moderate COVID-19 patients compared with levels in mild patients and healthy control (HC) subjects. *p<0.05, **p<0.01, ****p<0.0001 ns; non-significant.

Conflict of Interest statement:

The authors declare that there is no conflict of interest to this article.

Acknowledgments

We thank all the patients and their families who participated in this research. We thank all members of our laboratories for critical input and suggestions.
IMA is financially supported by the Welcome Trust (093080/Z/10/Z), the EPSRC (EP/T003189/1), and the Community Jameel Imperial College COVID-19 Excellence Fund (G26290) and by the UK MRC (MR/T010371/1). SM is supported by EU project 853850.

Authors Contributions:

EM designed the project and did experiments with NDR and NKD. HRJ approved all patients and advise all clinical aspects of study. HS, MM, MS, and SL provided all patients and demographic information of study participants. GF, JG, SM and IMA revised and comments all the paper. JG and IMA participated as corresponding authors.

References

1. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020;8(5):475-81.
2. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cellular & molecular immunology. 2020;17(5):533-5.
3. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clinical immunology. 2020:108427.
4. Mortaz E, Tabarsi P, Varahram M, Folkerts G, Adcock IM. The immune response and immunopathology of COVID-19. Frontiers in Immunology. 2020;11. doi.org/10.3389/fimmu.2020.02037
5. Archer SL, Sharp WW, Weir EK. Differentiating COVID-19 pneumonia from acute respiratory distress syndrome and high altitude pulmonary edema: therapeutic implications. Circulation. 2020;142(2):101-4.
6. Imai Y, Kuba K, Penninger JM. The renin–angiotensin system in acute respiratory distress syndrome. Drug Discovery Today: Disease Mechanisms. 2006;3(2):225-9.
7. Imai Y, Kuba K, Penninger JM. Angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Cellular and molecular life sciences. 2007;64(15):2006-12.
8. Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020;181(4):905-13. e7.
9. Graham R, Baric R, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan:An analysis based on decade-long structural studies of SARS 3 2020 J Virol . 2020 17;94(7):e00127-20.
10. Cuba K, Imai Y, Rao S, Gao H, Guo F. Guan Bet al. Un papel crucial de la enzima convertidora de angiotensina 2 (ACE2) en la lesión pulmonar inducida por coronavirus del SARS. Nat Med. 2005;11(8):875-9.
11. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme: cloning and functional expression as a captopril-insensitive carboxypeptidase. Journal of Biological Chemistry. 2000;275(43):33238-43.
12. Samavati L, Uhal BD. ACE2, much more than just a receptor for SARS-COV-2. Frontiers in cellular and infection microbiology. 2020;10:317.

13. Yeung ML, Teng JLL, Jia L, Zhang C, Huang C, Cai J-P, et al. Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system. Cell. 2021;184(8):2212-28. e12.
14. Iwasaki M, Saito J, Zhao H, Sakamoto A, Hirota K, Ma D. Inflammation triggered by sars-cov-2 and ace2 augment drives multiple organ failure of severe covid-19: Molecular mechanisms and implications. Inflammation. 2021 ;44(1):13-34
15. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive care medicine. 2020;46(4):586-90.
16. Chan KK, Dorosky D, Sharma P, Abbasi SA, Dye JM, Kranz DM, et al. Engineering human ACE2 to optimize binding to the spike protein of SARS coronavirus 2. Science. 2020;369(6508):1261-5.
17. Abd El-Aziz TM, Al-Sabi A, Stockand JD. Human recombinant soluble ACE2 (hrsACE2) shows promise for treating severe COVID19. Signal Transduction and Targeted Therapy. 2020;5(1):1-2.
18. World Health O. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. Interim guidance. Pediatria i Medycyna Rodzinna. 2020;16(1):9-26.
19. Díez-Pérez A, Sabaté RA, Petit I, Brasé A, Horcajada JP, Güerri-Fernández R, et al.Hospital-at-Home Expands Hospital Capacity During COVID-19 Pandemic. 2021. 22, 5, 939-942.
20. Gandhi R, Lynch J, Del Rio C. Mild or moderate COVID-19 . N Engl J Med 2020; 383:1757-1766.
21. Rahmanzade R, Rahmanzadeh R, Hashemian SM, Tabarsi P. Iran's Approach to COVID-19: Evolving Treatment Protocols and Ongoing Clinical Trials. Frontiers in Public Health. 2020;8:523.
22. Horby PW, Campbell M, Staplin N, Spata E, Emberson JR, Pessoa-Amorim G, et al. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): preliminary results of a randomised, controlled, open-label, platform trial. J Am Med Dir Assoc . 2021 May;22(5):939-942.
23. Borges do Nascimento IJ, von Groote TC, O'Mathúna DP, Abdulazeem HM, Henderson C, Jayarajah U, et al. Clinical, laboratory and radiological characteristics and outcomes of novel coronavirus (SARS-CoV-2) infection in humans: A systematic review and series of meta-analyses. PLoS One. 2020;15(9):e0239235.
24. Corey L, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. Science. 2020;368(6494):948-50.
25. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-α convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). Journal of Biological Chemistry. 2005;280(34):30113-9.

26. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, et al. Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. Proceedings of the National Academy of Sciences. 2008;105(22):7809-14.
27. Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. Journal of virology. 2010;84(2):1198-205.
28. Rieder M, Wirth L, Pollmeier L, Jeserich M, Goller I, Baldus N, et al. Serum ACE2, Angiotensin II, and Aldosterone Levels Are Unchanged in Patients With COVID-19. AJH. 2021;34(3):278-81.
29. Mostafa-Hedeab G. ACE2 as Drug Target of COVID-19 Virus Treatment, Simplified Updated Review. Reports of Biochemistry & Molecular Biology. 2020;9(1):97.
30. Zoufaly A, Poglitsch M, Aberle JH, Hoepler W, Seitz T, Traugott M, et al. Human recombinant soluble ACE2 in severe COVID-19. The Lancet Respiratory Medicine. 2020;8(11):1154-8.
31. Swärd P, Edsfeldt A, Reepalu A, Jehpsson L, Rosengren BE, Karlsson MK. Age and sex differences in soluble ACE2 may give insights for COVID-19. Critical Care. 2020;24(1):1-3.
32. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. Jama. 2020;323(16):1574-81.
33. Emilsson V, Gudmundsson EF, Aspelund T, Jonsson BG, Gudjonsson A, Launer LJ, et al. Serum levels of ACE2 are higher in patients with obesity and diabetes. Obes Sci Pract . 2021, 7, 2;239-243.
34. Zhang Y, Sun Y, Liu K, Alolga RN, Xu X, Feng G, et al. Low plasma angiotensin-converting enzyme 2 level in diabetics increases the risk of severe COVID-19 infection. Aging (Albany NY). 2021;13(9):12301.
35. Fagyas M, Kertész A, Siket IM, Bánhegyi V, Kracskó B, Szegedi A, et al. Level of the SARS-CoV-2 receptor ACE2 activity is highly elevated in old-aged patients with aortic stenosis: implications for ACE2 as a biomarker for the severity of COVID-19. GeroScience. 2021;43(1):19-29.
36. Kragstrup TW, Singh HS, Grundberg I, Nielsen AL-L, Rivellese F, Mehta A, et al. Plasma ACE2 predicts outcome of COVID-19 in hospitalized patients. PLoS One. 2021;16(6):e0252799.
37. Fließer E, Birnhuber A, Marsh LM, Gschwandtner E, Klepetko W, Olschewski H, et al. Dysbalance of ACE2 levels–a possible cause for severe COVID-19 outcome in COPD. J Pathol Clin Res . 2021 12;10.1002/cjp2.224.