Impact of the Angiotensin-Converting Enzyme (ACE) Inhibitors on the Course of the Acute Respiratory Distress Syndrome (ARDS) Developed During COVID-19 and Other Severe Respiratory Infections Under Hyperferritinemia Conditions: A Cohort Study

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ABSTRACT

BACKGROUND: Angiotensin-converting enzyme 2 (ACE2) is not only the entry route of SARS-CoV-2 infection but also triggers a major mechanism of COVID-19 aggravation by promoting a hyperinflammatory state, leading to lung injury, hematological and immunological dysregulation. The impact of ACE2 inhibitors on the course of COVID-19 is still unclear. The effect of ACE2 inhibitors on the course of acute respiratory distress syndrome (ARDS) during COVID-19 and other severe respiratory infections in conditions of hyperferritinemia (HF) was investigated.

METHODS: A cohort study of critically ill patients with COVID-19 and other respiratory diseases (widespread infection, pneumonia) who underwent treatment in The Critical Care Unit of the First University Clinic (Tbilisi, Georgia) during the 2020–2021 years was conducted. The impact of the ACE2 inhibitors on the course of the ARDS developed during COVID-19 and other severe respiratory infections in conditions of different severity of HF was evaluated.

RESULTS: In COVID-19-infected (I) and uninfected (II) patients with ARDS, ACE2 inhibitors reduce the levels of Ang II, C reactive protein (CRP) and D-dimer (I: from 1508.07 ± 26.68 to 48.51 ± 24.35, from 233.92 ± 13.02 to 198.12 ± 11.88, from 7.88 ± 0.47 to 6.28 ± 0.43; II: from 1000.14 ± 149.49 to 46.23 ± 88.21, 226.48 ± 13.81 to 183.52 ± 17.32, from 6.39 ± 0.58 to 5.48 ± 0.69) at moderate HF and Ang II, CRP levels (I: from 1845.89 ± 89.37 to 49.64 ± 51.05, from 209.28 ± 14.41 to 175.37 ± 9.84; II: from 1753.29 ± 65.95 to 49.76 ± 55.74, 287.10 ± 20.50 to 214.71 ± 17.32) at severe HF, reduce interleukin-6 (IL-6) expression at moderate HF (I: from 1977.23 ± 354.66 to 899.36 ± 323.76) and cause reduction of pCO_2 index at severe HF (I: from 69.80 ± 3.22 to 60.44 ± 2.20) in COVID-19-infected patients.

CONCLUSION: Study results show that the ACE2 inhibitors play an important role in the regulation of inflammatory processes in both COVID-19-infected and uninfected patients with ARDS. ACE2 inhibitors decrease immunological disorders, inflammation, and lung alveoli dysfunction, especially in COVID-19-infected patients.

KEYWORDS: ACE2 inhibitors, COVID-19, respiratory infections, hyperferritinemia

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Introduction

COVID-19 is characterized by heterogeneous clinical manifestations, complex pathophysiology, and a wide spectrum of the clinical picture. The main complication remains severe respiratory distress (ARDS) and further hypoperfusion syndrome. ARDS is a destructive lung injury from an uncontrolled inflammatory process that causes increased vascular permeability and capillary endothelial damage, causing diffuse alveolar destruction and leakage of protein-rich exudate, leading to progressive respiratory failure. ARDS is well-recognized as a major clinical problem with high morbidity and mortality rates.[1,2] COVID-19-associated ARDS is a possibly serious complication of COVID-19 that requires early detection and comprehensive management. The leading mechanisms of ARDS associated with COVID-19 include severe lung infiltration/edema and inflammation, which leads to disruption in alveolar homeostasis, changes in lung physiology, pulmonary fibrosis as well as endothelial inflammation, and vascular thrombosis.[3] COVID-19-associated ARDS is thought to have worse outcomes than ARDS from other causes.[2,4] Ventilation parameters for COVID-19-associated ARDS must be adapted to individual cases based on respiratory parameters, admissions, and

observation times. To properly assess the disease outcomes of COVID-19 with the highest accuracy, it's especially important to effectively combine the results of visual observations, clinical trial outcomes, and disease-specific parameters.

It is believed that SARS-CoV enters alveolar cells via a membrane-bound angiotensin-converting enzyme 2 (ACE2), which is the binding site for the coronavirus spike protein, promoting its adhesion to the cell surface, followed by internalization of the SARS-CoV/ACE2 complex, endosome formation, release of viral RNA and its subsequent transcription and replication to spread the infection.[5,6] ACE2 is the entry route of SARS-CoV-2 infection and triggers a major mechanism of COVID-19 aggravation by promoting a hyperinflammatory state in organs, leading to lung injury, hematological alterations, and immunological dysregulation. The association of ACE2 inhibitor drugs with COVID-19 disease is still uncertain.

The ACE isoform ACE2 unlike ACE, producing Angiotensin II (Ang II) with potent vasopressor effects, enhancing sympathetic tone, and revealing pro-inflammatory and mitogenic properties over the endothelial and epithelial cells, produces a heptapeptide called Ang 1–7 characterized vasodilatory, anti-inflammatory activity.[5,7] ACE2 is expressed on the surfaces of alveolar epithelial cells and vascular endothelial cells and plays an important role in blood pressure regulation.[8]

During SARS-CoV infection, virus-bound ACE2 is internalized into the cytoplasm which reduces ACE2 expression on the membrane surface,[9,10,11] and leads to the weakened ACE2-Ang (1-7)-MasR axis, mainly manifested by the increase of Ang II and decrease of vasodilator Ang (1-7) level,[12] and can cause dysregulation of vascular tone, blood pressure, inflammation of the endothelium, thrombosis, development of thrombotic complications and initiating life-threatening respiratory distress.

Table 1. Groups of studied patients.

COVID-19-infected patients	Ferritin level <1500 ng/mL	ACE2 inhibitor — yes (Group IA(+))
(Group I)	(Group IA)	ACE2 inhibitor — no (Group IA(-))
	Ferritin level >1500 ng/mL	ACE2 inhibitor — yes (Group I)
	(Group IB)	ACE2 inhibitor — no (Group IB(-))
COVID-19-uninfected	Ferritin level <1500 ng/mL	ACE2 inhibitor — yes (Group IIA(+))
Patients (Group II)	(Group IIA)	ACE2 inhibitor — no (Group IIA(-))
	Ferritin level >1500 ng/mL	ACE2 inhibitor — yes (Group IIB(+))
	(Group IIB)	ACE2 inhibitor — no (Group IIB(-))

It is well known that serum ferritin is an acute-phase reactant, reflecting the degree of acute and chronic inflammation in several systemic diseases. In addition, ferritin is also able to directly modulate the lymphocyte function and thus regulate the immune response. Ferritin represents a biomarker of disease progress and an independent predictor of various clinical outcomes in different patients.[13]

The study aimed to reveal the impact of the ACE2 inhibitors on the course of the ARDS developed during COVID-19 and other severe respiratory infections in conditions of hyperferritinemia (HF).

Methods

Study Design and Patient Selection

We conducted a cohort study, comparing outcomes of critically ill patients (212 patients (134 men (63.3%) and 78 women (36.7%)) with a mean age between 40 and 70 years) with COVID-19 and other respiratory diseases (widespread infection, pneumonia) associated with ARDS who underwent treatment in The Critical Care Unit of the First University Clinic (Tbilisi, Georgia) during the 2020–2021 years. Patients with COVID-19 were diagnosed by the positive result of the PCR test for SARS-CoV-2 RNA. The diagnosis of ARDS was based on the physical examination (severe shortness of breath or breathlessness, rapid and labored breathing), chest x-ray (to measure fluids in the lungs), and oxygen saturation levels (<50%). The severity of ARDS was assessed using the Berlin classification.

Inclusion and Exclusion Criteria. Inclusion criteria for the study were: age > 40 years, COVID-19 and other respiratory diseases (widespread infection, pneumonia) associated with respiration dysfunctions, ARDS, presence of HF, prior chronic exposure to ACE2 inhibitors, or no history of treatment with the ACE2 inhibitors.

Patients enrolled in the study were divided into four target groups (Table 1): Group I - COVID-19-infected patients, Group II - COVID-19-uninfected patients. Patients from studied groups I and II were divided according to ferritin concentrations in blood serum: moderate HF (<1500 ng/mL) - Groups IA, IIA and severe HF (>1500 ng/mL) – Groups IB, IIB. Patients from studied groups IA, IB, IIA, and IIB were divided according to the use of ACE2 inhibitors: with prior chronic exposure to ACE2 inhibitors - Groups IA(+), IIA(+), IB(+), and IIB(+), or without a history of treatment with the ACE2 inhibitors - Groups IA(-), IIA(-), IB(-), and IIB(-).

Laboratory Tests

The routine laboratory tests including biochemistry, coagulation function, and blood cells (platelets, leucocytes) count were performed in each patient. We evaluated also changes in

Table 2. Values of studied parameters in the patients' groups.

	Group I (COVID-19-infected)		Group II (COVID-19-uninfected)	
Ferritin < 1500ng/ml (A)	ACE2-(n)	ACE2 + (y)	ACE2-(n)	ACE2 + (y)
ANG II (pg/mL)	1508.07 ± 26.68*	48.51 ± 24.35**	1000.14 ± 149.49	46.23 ± 88.21**
CRP (mg/L)	233.92 ± 13.02	198.12 ± 11.88**	226.48 ± 13.81	183.52 ± 17.32**
Leukocytes ($\times 10^9$/L)	20.54 ± 1.60	16.07 ± 1.46**	19.50 ± 1.11	13.30 ± 1.33**
Platelets ($\times 10^3$µL)	76.70 ± 2.30*	64.33 ± 2.10***	69.70 ± 2.82	62.23 ± 3.37*
pCO_2 (mm Hg)	61.53 ± 3.38	61.80 ± 3.09	60.28 ± 2.76	56.78 ± 2.10
IL-6 (pg/µL)	1977.23 ± 354.66*	899.36 ± 323.76**	647.44 ± 241.01	807.77 ± 288.06
D-dimer (mg/L)	7.88 ± 0.47*	6.28 ± 0.43**	6.39 ± 0.58	5.48 ± 0.69**
Ferritin > 1500ng/ml (B)				
ANG II (pg/ml)	1845.89 ± 89.37*	49.64 ± 51.05**	1753.29 ± 65.95	49.76 ± 55.74**
CRP (mg/L)	209.28 ± 14.41*	175.37 ± 9.84**	287.10 ± 20.50	214.71 ± 17.32*
Leukocytes ($\times 10^9$/L)	20.25 ± 0.92*	16.63 ± 0.63**	16.87 ± 1.07	15.56 ± 0.90
Platelets ($\times 10^3$µL)	68.57 ± 4.17	66.40 ± 2.85	64.86 ± 4.28	49.85 ± 3.62**
pCO_2 (mm Hg)	69.80 ± 3.22*	60.44 ± 3.20**	58.04 ± 1.80	56.04 ± 1.52
IL-6 (pg/µL)	579.68 ± 209.43	631.43 ± 143.06	841.8 ± 136.91	751.00 ± 115.71
D-dimer (mg/L)	6.26 ± 0.87*	5.92 ± 0.59	5.96 ± 0.69	6.09 ± 0.58

* Statistically significant alterations of the parameters in COVID-19-infected and uninfected patients ($P < 0.05$);
** Statistically significant alterations of the parameters under the influence of ACE2 inhibitors ($P < 0.05$).

variables such as ANG II, Interleukin-6 (IL-6), C reactive protein (CRP), and pCO_2.

The level of Ang II in the blood was measured by the ELIZA method (Huma Reader HS device; Human ANGII ELISA Kit reagent); IL-6 concentration in the blood - by the electrochemiluminescence (ECL) method (Cobase 411 (Roche) device; Elecsys IL-6 reagent); CRP was determined by spectrophotocolorometric method (Cobas C 111 (Roche) analyzer, Cobas 111 CRP reagent); D dimer - with immunofluorescence method (Fincare III Plus device); $pC0_2$ by radiometric cartridge method (RADIOMETER device).

Ethics Statement

All precautions were taken to protect the patient's privacy, and the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental health and the personality of the patients. The research protocol contained a statement of the ethical considerations and indicated that there is compliance with the principles enunciated in this Declaration. All medical evaluations were performed by scientifically qualified technicians and under the supervision of clinically qualified physicians. Written informed consent was obtained from all eligible patients before enrollment in our study. The study was approved by the Ethics Committee of Tbilisi State Medical University.

Statistical Analysis

Analysis of variance (ANOVA) was used for statistical analysis. We used the Software Program SPSS-12 for Windows to process the data and visualize the results.

Results

COVID-19-infected patients with ARDS with moderate HF (ferritin <1500 ng/mL) with no prior ACE2 inhibitors history (Group IA(-)) had the initial level of Ang II higher than COVID-19-uninfected individuals (Group IIA(-)) ($F = 4.8$; $p = 0.045$). In patients with extreme HF (ferritin >1500 ng/mL) (Groups IB(-), IIB(-)) this difference was diminished ($F = 0.6$, $p = 0.45$). The level of Ang II significantly decreased in ACE2 inhibitor users' groups (Groups IA(+), IIA(+), IB(+), IIB) (+) ($F = 488$, $p < 0.001$; $F = 468$, $p < 0.001$) (Table 2, Figure 1).

Leukocytes count did not differ in COVID-19-infected and uninfected patients with ARDS (Groups IA, IIA) when the ferritin concentration was <1500 ng/mL [ACE2 inhibitor (+) $F = 1.89$; $p = 0.18$; ACE2 inhibitor(-) $F = 0.19$; $p = 0.66$]. In the setting of extreme HF (ferritin >1500 ng/mL) in patients with ARDS who didn't use ACE2 inhibitors, leukocytes level

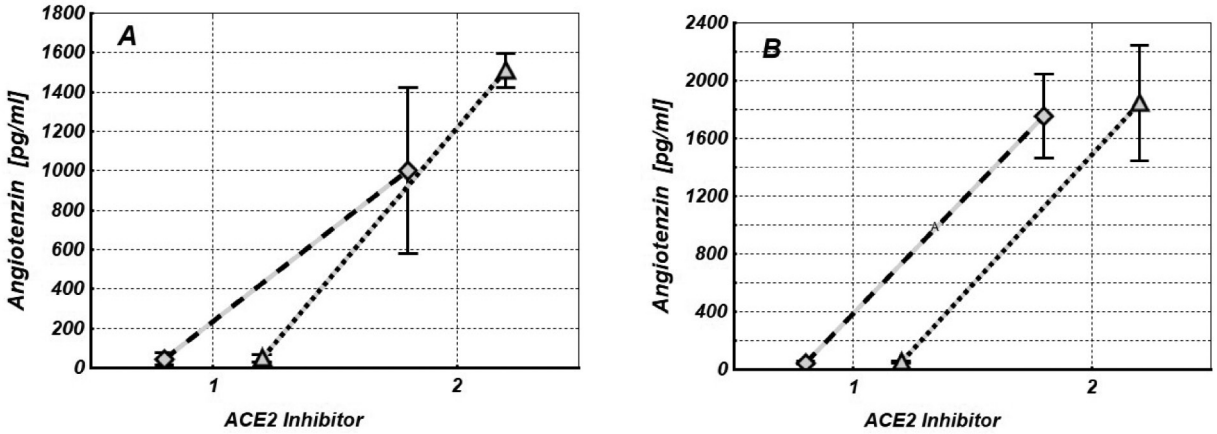

Figure 1. Angiotensin II levels (control level: 31.25-2000pg/mL) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE2 inhibitors use (A - ferritin level <1500 ng/mL; B - ferritin level >1500 ng/mL) (Δ- COVID-19-infected patients; ◊ - COVID-19-uninfected patients).

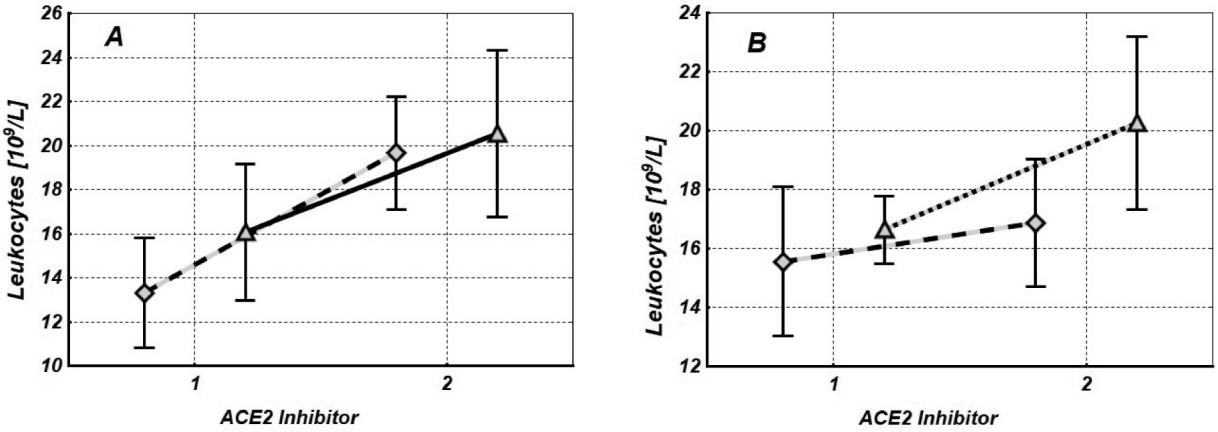

Figure 2. Leukocyte levels (control level: 4.00–11.00 × 10^9/L) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE2 inhibitors use (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL). (Δ-COVID-19-infected patients; Δ - COVID-19-uninfected patients).

was higher in COVID-19-infected patients (Group IB(-)) [ACE2 inhibitor(-) F = 4.58; p = 0.05]. however, in the patients' group, who used ACE2 inhibitors (Group IB(+)) difference in leukocyte level was not detected [ACE2 inhibitor(+) F = 1.05; p = 0.31].

ACE2 inhibitors cause a reduction in inflammatory marker, leukocytes count, in COVID-19-infected [F = 4.25; p = 0.05] and uninfected patients [F = 14.56; p = 0.001] when ferritin concentration was <1500 ng/mL (Groups IA(+), IIA(+)) and in COVID-19-infected patients with ferritin >1500 ng/mL (Group IB(+)) [F = 10.33; p = 0.004] (Table 1, Figure 2).

IL-6 level in COVID-19-infected patients was higher than in uninfected patients without prior use of ACE2 inhibitors when the ferritin concentration was <1500 ng/mL (Groups IA(-), IIA(-)) [ACE2 inhibitor(-) F = 10.95; p = 0.003]. In the setting of extreme HF (ferritin >1500 ng/mL) IL-6 level was not statistically important different in COVID-19-infected and uninfected patients (Groups IB, IIB) [ACE2 inhibitor(-) F = 1.78; p = 0.21; ACE2 inhibitor(+) F = 0.006, p = 0.93]. ACE2 inhibitors decreased IL-6 levels in COVID-19-infected patients, when ferritin concentration was <1500 ng/mL (Group IA(+)) [F = 5.03; p = 0.03] and did not affect the level of IL-6 in all other patients' groups [ferritin >1500 ng/mL; COVID-19(+) F = 0.35; p = 0.55; COVID-19(-) F = 0.25; p = 0.62] (Table 2, Figure 3).

In patients infected with COVID-19, without prior use of ACE2 inhibitors (Group I(-)), platelets count was higher than in those without COVID-19 (Group II(-)) when the ferritin concentration was <1500 ng/mL [F = 4.92; p = 0.037]. In the setting of extreme HF (ferritin >1500 ng/mL), platelets count was not statistically important difference in COVID-19-infected and uninfected patients without prior use of ACE2 inhibitors [F = 0.18; p = 0.67] (Groups IB(-), IIB(-)). Chronic use of ACE2 inhibitors lowered platelets count in COVID-19-infected and uninfected patients when the ferritin concentration was <1500 ng/mL (Groups IA(+),

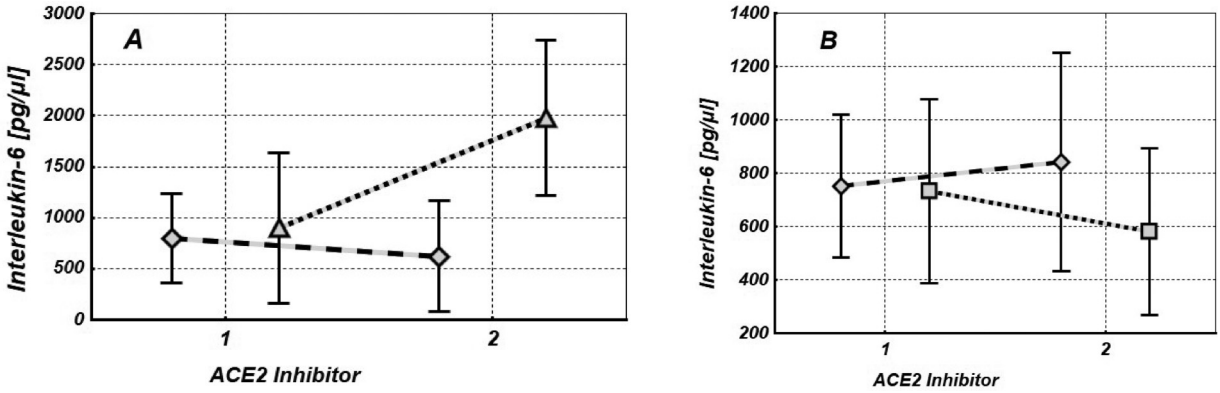

Figure 3. Levels of IL-6 (control level: 1000–7000pg/µL) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE inhibitors use (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL). (Δ- COVID-19-infected patients; ◊ - COVID-19-uninfected patients).

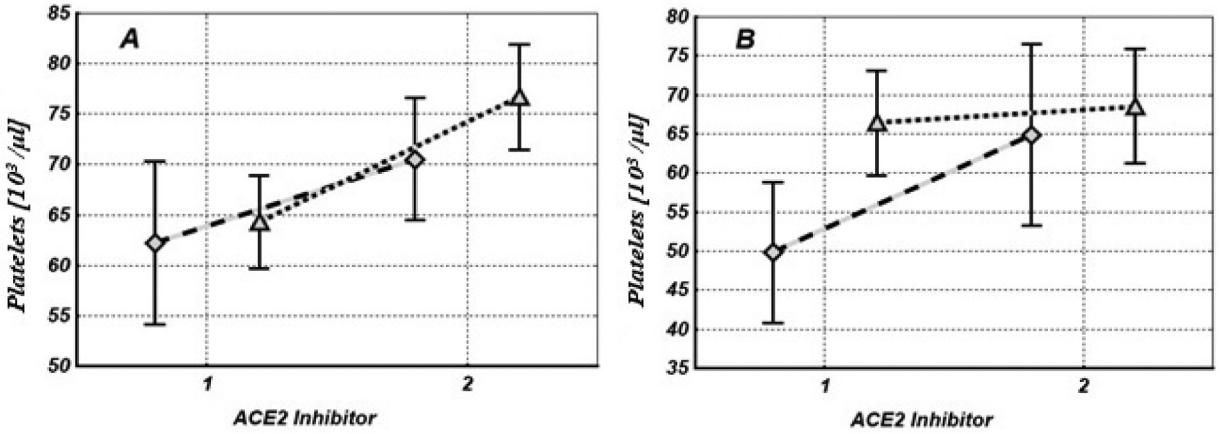

Figure 4. Platelets count (control level: 150–380 × 10^3µL) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE2 inhibitors use (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL). (Δ- COVID-19-infected patients; ◊ - COVID-19-uninfected patients).

IIA(+)) [COVID-19(+) F = 15.72; p = 0.001; COVID-19(-) F = 4.7128; p = 0.031], and in COVID-19-uninfected patients with extreme HF (ferritin >1500ng/ml) (Group IIB(+)), but had no effect in COVID-19-infected patients with extreme HF (GroupIB(+)) [F = 7.15; p = 0.02] (Table 2, Figure 4).

In patients infected with COVID-19, D-dimer levels appear to be higher than in those without COVID-19 when the ferritin concentration was <1500 ng/mL [F = 4.1142; p = 0.05]; while in the setting of extreme HF (ferritin >1500 ng/mL), D-dimer level was not statistically importantly different in COVID-19-infected and uninfected patients [F = 0.02; p = 0.88] (Groups IB(-), IIB(-)). Chronic ACE2 inhibitors use was associated with a slight lowering of D-dimer levels in COVID-19-infected [F = 6.26; p = 0.02] and uninfected patients [F = 2.1; p = 0.07] when the ferritin concentration was <1500ng/ml (Groups IA(+), IIA(+)), but there was no notable difference in D-dimer levels between COVID-19-infected and uninfected patients with extreme HF(Groups IB(+), IIB(+)) [COVID-19(-) F = 0.02; p = 0.88; COVID-19(+) F = 0.19; p = 0.75] (Table 2, Figure 5).

CRP levels did not differ between COVID-19-infected and uninfected patients when the ferritin concentration was <1500ng/ml [ACE2 inhibitor(+) F = 0.57; p = 0.45; ACE2 inhibitor (-) F = 0.14; p = 0.71]; CRP levels were significantly higher in COVID-19-uninfected patients compared to COVID-19-infected patients in the setting of extreme HF [ACE2 inhibitor(+) F = 4.19 p = 0.05 and ACE2 inhibitor(-) F = 11.85; p = 0.006]. ACE2 inhibitors cause the reduction in inflammatory markers - CRP in patients in all groups [COVID-19 (-) F = 3.8; p = 0.05 and COVID-19(+) F = 4.12; p = 0.045; ferritin <1500ng/ml]; and [COVID-19(-) F = 7.2711; p = 0.022; COVID-19(+) F = 3.77; p = 0.050; ferritin >1500 ng/mL] (Table 2, Figure 6).

There were no notable changes in pCO_2 levels in COVID-19-infected and uninfected patients without prior chronic ACE2 inhibitor use in the setting of moderate HF

(ferritin <1500 ng/mL) (Groups IA(-), IIA(-)), in these patients groups, ACE2 inhibitors had no notable effect on the pCO_2 levels.

In conditions of extreme HF level (ferritin >1500 ng/mL) in COVID-19-infected patients' blood pCO_2 levels were statistically significantly higher than in COVID-19-uninfected patients [$F = 9.48$; $p = 0.01$], in this group of patients ACE2 inhibitors induced to decrease in pCO_2 level $F = 5.75$; $p = 0.02$] (Table 2, Figure 7).

Discussion

ACE2 was identified to be the cell receptor of SARS-CoV-2,[14] therefore, ACE2 distribution and expression in the human body may represent the possible routes of COVID-19 infection. High ACE2 expression was recognized in type II alveolar cells of the lungs, oral mucosa, myocardial cells, kidney proximal tubule cells, and bladder urothelial cells.[15,16] It was demonstrated that alveolar epithelial type II cells, the main pathway for viral invasion, represent about 83% of ACE2-expressing cells.[8]

After binding of SARS-CoV-2 to the ACE2 receptor, both SARS-CoV-2 and ACE2 are internalized in a cell by endocytosis, so that surface ACE2 is then downregulated, resulting in unopposed Ang II accumulation.[17] Acting via the type 1 Ang II receptor (AT1), Ang II induces the production of reactive oxygen species (ROS) by activation of NADP(H) oxidases,[18,19] initiates an inflammatory cascade by reduced nicotinamide-adenine dinucleotide phosphate oxidase, and nuclear factor-κB, which mediates transcription and proinflammatory gene expression and increases ROS, adhesion molecules and chemokines levels, having a pro-inflammatory effect on leucocytes, endothelial, and vascular smooth muscle cells. An excess of ROS decreases nitric oxide (NO) bioavailability and causes vasoconstriction. Moreover, Ang II interrupts the anti-inflammatory effects of insulin. Together, these effects promote a prothrombotic state as well as plaque rupture.[20] ACE2 down-regulation and elevation of Ang II level in severe COVID-19 patients can be crucial factors in excessive cytokine release and pro-thrombotic activation.

According to our study results, in patients infected with COVID-19 who did not receive ACE2 inhibitors, the initial level of Ang II was higher than in uninfected patients (Table 2, Figure 1); accordingly, the content of leukocytes, platelets, D-dimer and IL-6 was higher in COVID-19-infected patients compared to their levels in COVID-19-uninfected critical patients (Figures 2 to 5). Recent studies suggest that Ang II promotes thrombosis through increased platelet activation induced by T cell-dependent IL-6 signaling.[21,22] A high level of IL-6 contributes to hypercoagulation by enhancing platelet production and activation, promoting an imbalance between plasma levels of coagulative and anti-coagulative factors, and the development of endothelial dysfunction. Hence, the elevated serum levels of pro-inflammatory cytokines in severe COVID-19 participate in Ang II-mediated thrombosis and vascular injury.

One of the properties of ACE2 inhibitors is their ability to regulate cytokines production (Figure 3), which is probably related to the inhibition of Ang II formation. It is believed that inhibition of Ang II by ACE2 inhibitors plays an important role in the regulation of immune system functions by modifying T-cell populations.[23] It has been demonstrated that ACE2 inhibitors decrease Ang II-induced production of proinflammatory cytokines and chemokines.[24] According to the results of our studies, ACE2 inhibitors reduced levels of inflammatory markers (leukocyte, IL-6 (Figures 2 and 3), CRP content (Figure 6)) in COVID-19-infected and uninfected patients with ARDS, regulated the functioning of the blood coagulation system, that revealed in the decrease of the platelets and D-dimer levels in the blood of COVID-19-infected patients with ARDS (Figures 4 and 5), and by this way

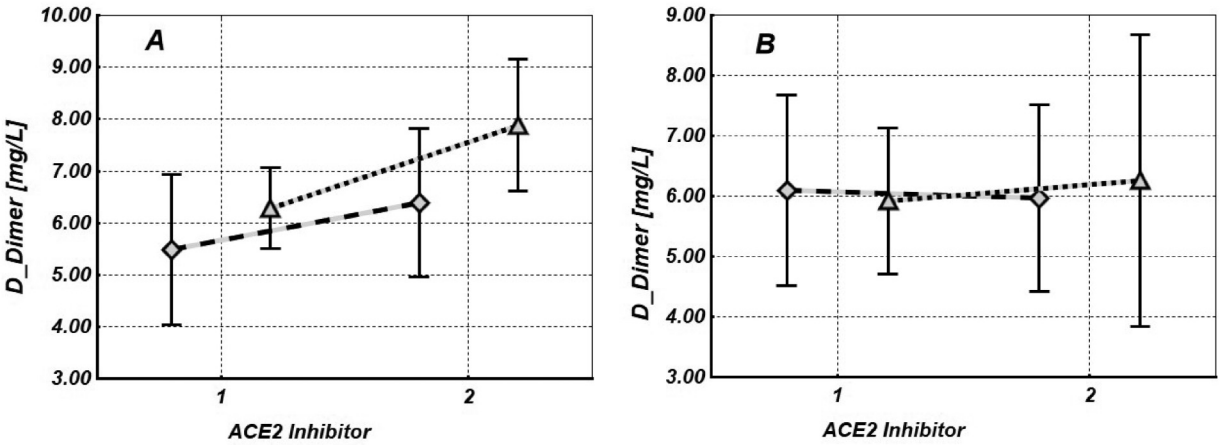

Figure 5. D-dimer level (control level: 0.10–0.50mg/L) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE2 inhibitors use (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL). (∆- COVID-19-infected patients; ◊ - COVID-19-uninfected patients).

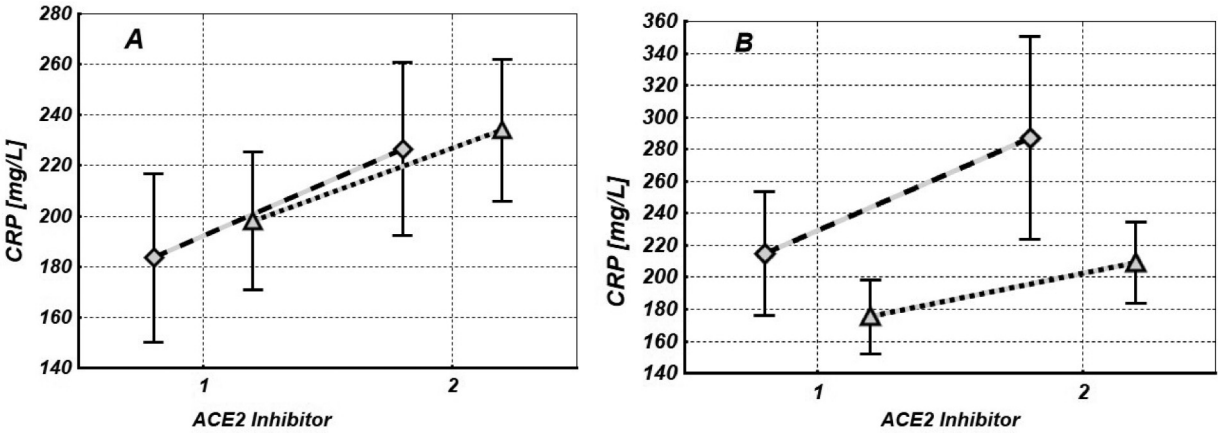

Figure 6. CRP levels (control level: < 5mg/L) in COVID-19-infected and uninfected patients with (1) or without (2) prior ACE2 inhibitors use (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL). (Δ- COVID-19-infected patients; ◊ - COVID-19-uninfected patients).

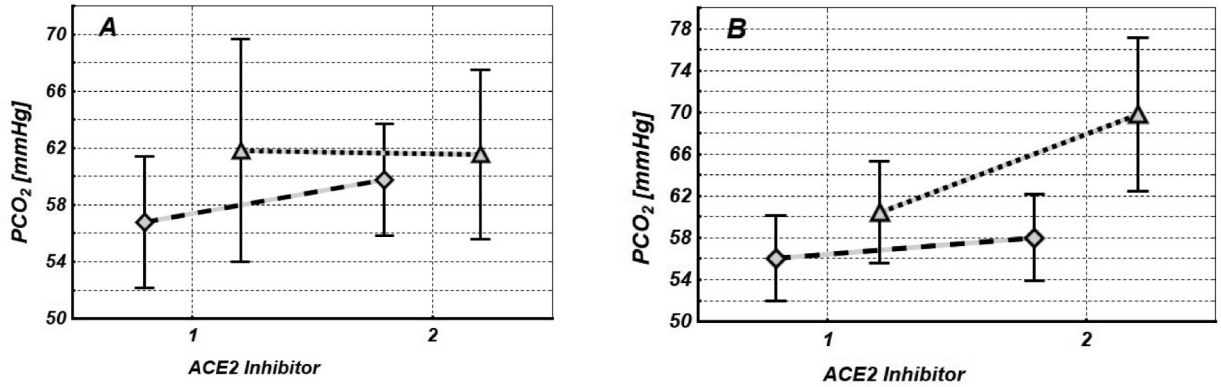

Figure 7. pCO_2 levels (control level: 32–45mm Hg) in COVID-19-infected and uninfected patients with (1) or without (2) prior use of ACE2 inhibitors (A - ferritin level <1500ng/mL; B - ferritin level >1500ng/mL)). (Δ- COVID-19 infected patients; ◊ - COVID-19-uninfected patients).

controlled systemic inflammation. As a result, the risk of vascular thrombosis in patients with ARDS was reduced. It is noteworthy the decrease of pCO_2 levels in the blood of COVID-19-infected patients against the background of ACE2 inhibitors (Figure 7), which indicates the improvement of lung function.

Ferritin is known as a molecule storing iron ions, a dynamic "buffer" of iron, maintaining its availability. Cellular ferritin values are regulated at the translational level by the iron-regulatory proteins/iron-responsive elements (IRP/IRE) system, which is dependent on the amount of iron in the body.[25] In cells, ferritin can be localized in the cytoplasm, nucleus, and mitochondria. The existence of ferritin also was found in erythrocytes and blood serum.[26,27,28] In response to the inflammatory state, cells produce large amounts of ferritin. Still is not clear whether ferritin is only a sign of disease progression or has a modulating role in the pathogenesis of the disease. Over the last few years, accumulated data implicate a role for ferritin as a signaling molecule and direct mediator of the immune system. It is believed that in response to virus-induced injury, cytokines (IL-1β, IL-6, and IFN-γ) stimulate the production of protective proteins in the liver, including ferritin and CRP.[29] During viral infections the immune system of the body supports increased serum ferritin levels in the cells, limiting the availability of iron to pathogens (necessary for their proliferation).[30,31] As a result, the concentration of iron in the systemic circulation is decreased, and the level of serum ferritin increased (which was recorded in COVID-19).

Ferritin, by activation of NF-?B, promotes excessive production of ROS and lipid peroxidation, the release of pro-inflammatory mediators leading to extensive damage of cells and tissues, and cell apoptosis, directly modulates the function of lymphocytes and, thus, regulates innate immunity, enhances the inflammatory response. and initiates a vicious circle.[32,33] The concentration of ferritin in the cells is about 1000 times higher than in the serum; therefore, the increase of serum ferritin can be the result of cellular stress and cell lysis.[34,35,36] In addition to damaged cells, macrophages are also a source of increased ferritin levels.[37,38]

Possibly, during COVID-19-induced hyperinflammation, high blood ferritin, as a prooxidative agent and immune response modulator, initiates a cycle of destructive events, which can cause additional lesions in tissues. Worse prognosis in critically ill patients during a multitude of clinical conditions, including COVID-19, is associated with HF. Therefore, ferritin is crucial in COVID-19 outcomes.[39,40] It was proposed that the exceptionally high ferritin levels observed in clinical conditions are not just the product of the inflammation but may contribute to developing a cytokine storm.[41] The role of ferritin in the pathophysiology of COVID-19 is not fully understood; the alterations of ferritin levels in patients with severe COVID-19 and high mortality rates in patients with HF levels need further investigation.

Our study results show that in COVID-19-infected patients with ARDS at HF conditions, the level of Ang II was especially high (Figure 1), at the same time, the pCO_2 level in blood also increased (Figure 7). These data indicate the link between elevated Ang II and ferritin levels, inflammation, and lung alveoli dysfunction in COVID-19-infected critically ill patients.

Ang II participates in the induction of iron metabolism-related gene expression, including hepcidin,[42] which is a key regulator controlling the delivery of iron to blood plasma from intestinal cells, erythrocyte-recycling macrophages, and iron-storing hepatocytes. Secretion of hepcidin can be increased during inflammatory states, including COVID-19,[43] and IL-6 is the necessary and sufficient cytokine for hepcidin induction during inflammation.[44] At the same time due to the similarity of the hepcidin and part of the spike glycoprotein structure of the SARS-CoV-2 virus, it is hypothesized that this glycoprotein could have a hepcidin-like effect[37] and, therefore, self-increase cellular and serum concentrations of ferritin, regardless of the degree of inflammatory effects, with all attendant consequences.

Conclusion

Based on our study results we can conclude, that ACE2 inhibitors play an essential role in regulating inflammatory processes in both COVID-19-infected and uninfected patients with ARDS.

In COVID-19-infected and uninfected patients with ARDS, ACE2 inhibitors reduce the levels of Ang II and CRP, activity of leukocytes, and blood pro-coagulation system (level of D dimer) at moderate and severe HF conditions. ACE2 inhibitors reduce the expression of pro-inflammatory cytokines (IL-6) in conditions of moderate HF and cause a reduction of the pCO_2 index in conditions of severe HF in COVID-infected patients with ARDS. Therefore, ACE2 inhibitors can decrease the quality of inflammation, immunological disorders, and lung alveoli dysfunction in critically ill (especially COVID-19-infected) patients.

In conditions of moderate HF severity course of ARDS is higher in COVID-19-infected patients.

Study results show that severe HF in patients with ARDS can be considered an indicator of the severity of the disease and is associated with a high risk of mortality.

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None

Author contribution(s)

Magda Rurua: Conceptualization; Methodology; Project administration.
Elena Pachkoria: Conceptualization; Resources.
Tamar Sanikidze: Writing – review & editing.
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Ethics Approval and Consent to Participate

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All experimental protocols were approved by the Ethics Committee of Tbilisi State Medical University, on 12 June 2020 (meeting #4-2020/81. Informed consent was obtained from each patient and voluntary participant. All studies were performed according to the guidelines and regulations of the Declaration of Helsinki.

Conflict of Interest Statement

No

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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