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Plasmatic renin-angiotensin system in normotensive and hypertensive patients hospitalized with COVID-19

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ABSTRACT

Background: Besides its counterbalancing role of the renin-angiotensin system (RAS), angiotensin-converting enzyme (ACE) 2 is the receptor for the type 2 coronavirus that causes severe acute respiratory syndrome, the etiological agent of COVID-19. COVID-19 is associated with increased plasmatic ACE2 levels, although conflicting results have been reported regarding angiotensin (Ang) II and Ang-(1–7) levels. We investigated plasmatic ACE2 protein levels and enzymatic activity and Ang II and Ang-(1–7) levels in normotensive and hypertensive patients hospitalized with COVID-19 compared to healthy subjects.

Methods: Ang II and Ang-(1–7), and ACE2 activity and protein levels were measured in 93 adults (58 % (n = 54) normotensive and 42 % (n = 39) hypertensive) hospitalized with COVID-19. Healthy, normotensive (n = 33) and hypertensive (n = 7) outpatient adults comprised the control group.

Results: COVID-19 patients displayed higher ACE2 enzymatic activity and protein levels than healthy subjects. Within the COVID-19 group, ACE2 activity and protein levels were not different between normotensive and hypertensive-treated patients, not even between COVID-19 hypertensive patients under RAS blockade treatment and those treated with other antihypertensive medications. Ang II and Ang-(1–7) levels significantly decreased in COVID-19 patients. When COVID-19 patients under RAS blockade treatment were excluded from the analysis, ACE2 activity and protein levels remained higher and Ang II and Ang-(1–7) levels lower in COVID-19 patients compared to healthy people.

Conclusions: Our results support the involvement of RAS in COVID-19, even when patients under RAS blockade treatment were excluded. The increased circulating ACE2 suggest higher ACE2 expression and shedding.

1. Introduction

Angiotensin-converting enzyme (ACE) 2 is a counterbalancing enzyme of the renin-angiotensin system (RAS) that converts angiotensin (Ang) II into Ang-(1–7), whose effects oppose the effects of Ang II via the AT1 receptor [1]. The soluble, enzymatically active form of ACE2 (sACE2) is generated by ACE2 cleavage in the plasma membrane by ADAM17 in response to inflammatory signals [2,3]. In addition to its balancing role in the RAS, ACE2 is the receptor for the type 2 coronavirus that causes severe acute respiratory syndrome, the etiological agent of COVID-19 [4,5]. COVID-19 severity has been associated with

elevated sACE2 levels, male sex, and diabetes, as well as increased Ang II and Ang-(1–7) levels [6–9]. In contrast, others reported no differences in Ang II and Ang-(1–7) levels and sACE2 between patients with and without COVID-19 [10,11]. One study showed increased plasmatic sACE2 in non-severe COVID-19 patients under ACE inhibitor (ACEI) treatment, though the sample investigated was small [10]. An increased sACE2 may reflect higher ACE2 expression, ACE2 shedding or both in those patients. However, we have recently shown that RAS blockade did not modify ACE2 protein expression in human type II pneumocytes, which are key cells for lung homeostasis, of subjects under ACEI treatment [12]. Here, we evaluated plasmatic sACE2 protein levels and

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enzymatic activity and Ang II and Ang-(1–7) levels in patients hospitalized with COVID-19 compared to healthy subjects. We also compared these variables in normotensive and hypertensive COVID-19 patients.

2. Material and methods

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

2.1. Ethics statement

This study was a prospective study of de-identified material, thus informed consent was required. In those patients requiring mechanical ventilation informed consent was obtained from a close relative of the patient. Approval for the study was obtained from the Ethics and Clinical Research Committee of Hospital San Martín de La Plata (HSMLP2020/0028) and Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (RESCD-2020–330). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association and with the principles of the Helsinki Declaration.

2.2. Human samples

Ninety-three adults with a positive reverse transcription-polymerase chain reaction test for COVID-19 via a standard-of-care nasopharyngeal swab who required hospitalization in Hospital San Martín de La Plata were prospectively enrolled. Inclusion criteria included patients who were older than 18 years and were hospitalized after being diagnosed with COVID-19 infection in the general ward of Hospital San Martín de La Plata. Severity of disease was graded by the maximum requirement for respiratory support as severe (requiring invasive, mechanical ventilation at least once during the disease course) versus moderate (requiring simple face or reservoir mask). Pregnant women and those refused to participate in the study were excluded. Deidentified samples from healthy, normotensive ($n = 33$) and hypertensive ($n = 7$) volunteers' subjects comprised the control group. Inclusion criteria for healthy subjects was an age over 18 years with no previous SARS-CoV-2 infection. All the subjects were required acceptance and signing of the informed consent to participate in the study.

Plasma collection was performed under an institutional review approval after informed consent. Blood samples were obtained the first day the patient was admitted to general ward for hospitalization. Whole blood was collected into EDTA-containing tubes with protease inhibitors for Ang II and Ang-(1–7) measurement and a separate tube for ACE2 activity and protein levels measurement. Those patients that do not have all the measured parameters were not included in the analysis.

2.3. Ang II and Ang-(1–7) levels measurement

Blood samples were collected in EDTA-tubes containing 0.44 mmol/L o-phenanthroline, 1 mmol/L Na+para-chloromercuribenzoate and 25 mmol/L EDTA. Samples were centrifuged at 3000 xg for 20 min at 4°C and plasma were loaded into SepPak C18 cartridges for angiotensins extraction as previously described [13,14]. Each sample was corrected for each recovery. Angiotensin levels were quantified by radioimmunoassay using angiotensins labelled in our laboratory as previously described [15]. Radioimmunoassay for Ang-(1–7) has been previously validated [15]. Limit of detection of Ang II and Ang-(1–7) assays were 4 pg/mL and 20 pg/mL, respectively. Intra-assay and inter-assay variability were $13.7 \pm 2.3 \%$ and $12.4 \pm 3.1 \%$, respectively.

2.4. ACE2 enzymatic activity

ACE2 activity was measured using an ACE2-quenched fluorescent substrate (Mca-Ala-Pro-Lys(Dnp)-OH; Enzo Life Sciences) as previously described [16]. Specific activity of ACE2 was determined by addition of

Table 1

Baseline characteristics of the investigated population.

	Healthy subjects	Hospitalized COVID-19 patients	P value
Number of subjects, n	40	93	
Age, $\bar{X} \pm SD$	48	54 \pm 16	0.07
	\pm 17		
Female, n (%)	21 (53)	32 (33)	0.016
Hypertension, n (%)	7 (18)	41 (44)	0.004
Diabetes, n (%)	0 (0)	24 (26)	
Chronic kidney disease, n (%)	0 (0)	10 (11)	
Ischemic cardiomyopathy, n (%)	0 (0)	8 (9)	
Smoking, n (%)	0 (0)	7 (8)	
Obesity, n (%)	0 (0)	5 (5)	
Chronic obstructive pulmonary disease, n (%)	0 (0)	4 (4)	
Stroke, n (%)	0 (0)	2 (2)	
Dyslipemia, n (%)	0 (0)	2 (2)	

To evaluate differences by age, Student *t*-test for independent variables was applied because the variable age followed normal distribution and there was variance homogeneity. In the case of the sex variable, since it was categorical, the Chi-Square Test was applied. Variables were independent.

the ACE2 inhibitor MLN4760 (10 μ mol/L). Results were expressed as RFU (relative fluorescent units)/mL sample/min.

2.5. ACE2 and interleukin (IL) levels

ACE2 and ILs levels were measured by commercially available sandwich enzyme-linked immunosorbent assays (ELISAs) following manufacturer's instructions. The following ELISA kits were employed:

Table 2

Baseline characteristics of the hospitalized COVID-19 patients.

	Normotensive COVID-19 patients	Hypertensive COVID-19 patients	P value
Number of subjects, n	54	39	
Age, $\bar{X} \pm SD$	49 \pm 17	60 \pm 13	<0.001
Female, n (%)	21 (39)	11 (28)	0.248
Systolic blood pressure (mmHg, IQR)	120 (20)	136 (30)	0.003
Diastolic blood pressure (mmHg, IQR)	80 (10)	80 (10)	0.802
Death, n (%)	4 (7)	3 (8)	0.856
ICU, n (%)	6 (11)	6 (15)	0.569
ACEI-treated	0	23 (59)	
ARB-treated	0	4 (10)	
Amlodipine-treated	0	16 (41)	
Carvedilol-treated	0	4 (10)	
Atenolol-treated	0	4 (10)	
HCTZ-treated	0	1 (3)	
Diabetes, n (%)	7 (13)	17 (44)	0.001
Chronic kidney disease, n (%)	5 (9)	5 (13)	0.539
Ischemic cardiomyopathy, n (%)	2 (4)	6 (15)	0.065
Smoking, n (%)	5 (9)	2 (5)	0.467
Obesity, n (%)	2 (4)	3 (8)	0.413
Chronic obstructive pulmonary disease, n (%)	2 (4)	2 (5)	0.817
Stroke, n (%)	1 (2)	1 (3)	0.758
Dyslipemia, n (%)	1 (2)	1 (3)	0.758

ICU: intensive care unit.

To evaluate differences by age, Student *t*-test for independent variables was applied because the variable age followed normal distribution and there was variance homogeneity. In the case of the sex variable, since it was categorical, the Chi-Square Test was applied. Variables were independent. The variables systolic and diastolic blood pressure did not follow normal distribution and the Mann-Whitney *U*-test was applied. For differences in comorbidities, Difference Between Two Proportions Test was applied.

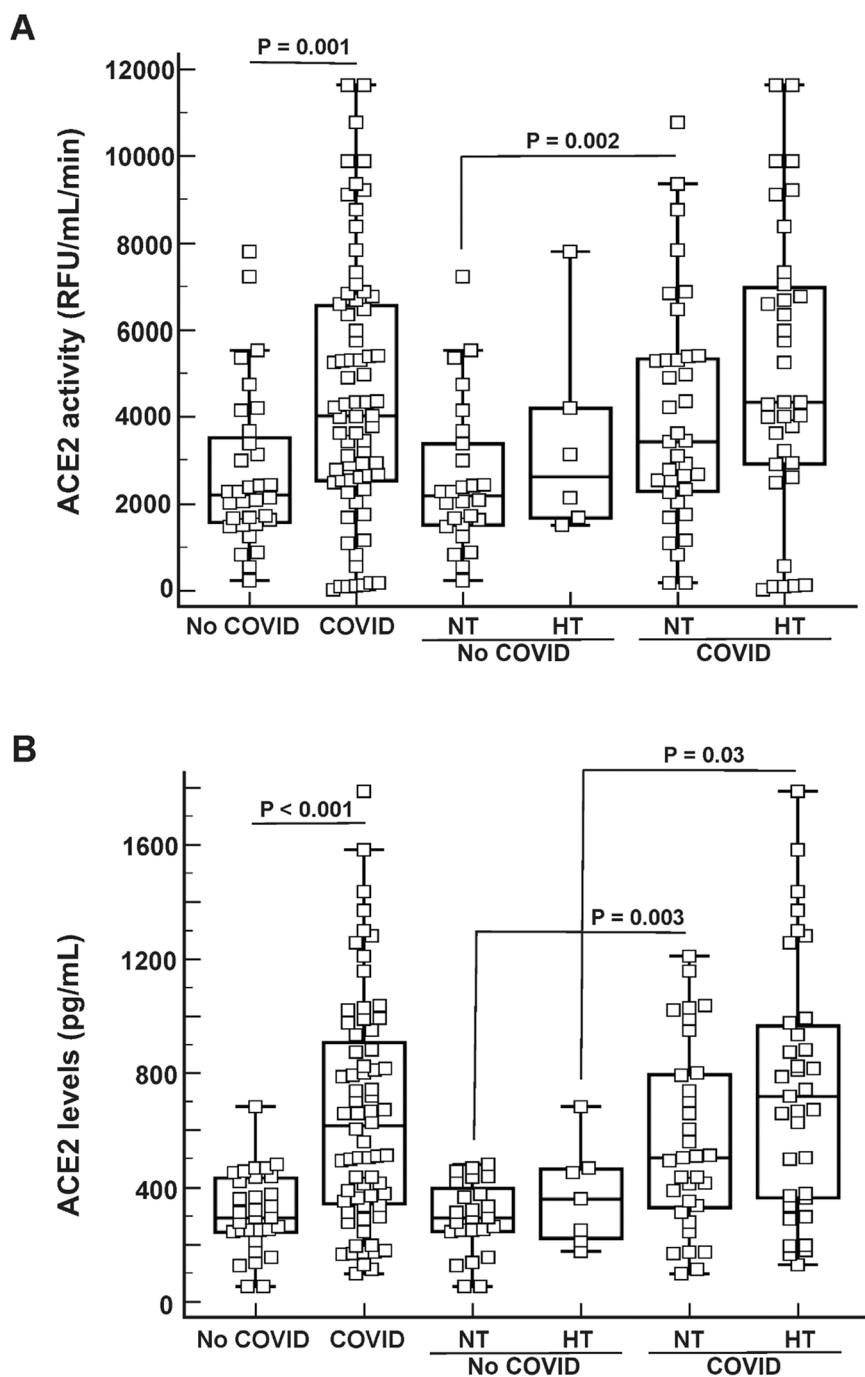


Fig. 1. sACE2 activity and protein levels in healthy subjects and hospitalized COVID-19 patients: (A) sACE2 activity and (B) sACE2 protein levels in the plasma of healthy subjects (no COVID, $n = 40$) and hospitalized COVID-19 patients (COVID, $n = 93$); non-hypertensive (NT no COVID, $n = 33$) and hypertensive (HT no COVID) healthy subjects ($n = 7$); and non-hypertensive (NT COVID, $n = 54$) and hypertensive (HT hospitalized COVID-19 patients ($n = 39$)). The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the boxes show the median values and the whiskers extending from both ends of the boxes are minimum and maximum values. Wilcoxon Test (Mann-Whitney U) test was applied for statistical analysis.

human IL-6 (BD Biosciences cat. 555220); human IL-8 (BD Biosciences cat. 555224); human IL-1 β (BD Biosciences cat. 557953); human IL-10 (BD Biosciences cat. 555157), and human ACE2 duoset kit (R&D SYSTEMS cat. DY933-05).

2.6. Statistical analysis

The data were analyzed by a statistician (M.N.) using SPSS Statistics 19 software. Kolmogorov–Smirnov test was applied to verify normal distribution. Levene test was applied to verify variance homogeneity. If the assumptions were met, the Student's Test was applied for independent samples, otherwise the Wilcoxon Test (Mann-Whitney U) was applied for independent samples. The statistical power of mathematical analyses (GPower software, Wilcoxon test) was of 80 % with $\alpha = 0.05$

and a size effect of 0.5. Spearman correlation analysis was applied to investigate correlations. $P < 0.05$ was considered statistically significant. Since antihypertensive treatment with RAS inhibitors may influence the results, a secondary analysis was performed excluding patients under treatment with ACEIs or Ang receptor blockers (ARBs).

Potential confounders may be sex and age. Sex did not influence the data. Age was significantly different only in normotensive vs hypertensive COVID-19 patients. Because the variables ACE2 activity and ACE2, Ang II and Ang-(1–7) levels did not follow a normal distribution, non-parametric 2-factor ANOVA (Kruskal Wallis) was applied. The two factors were hypertension (with two levels, normotensive, and hypertensive) and age (with two levels, people younger than 60 years old and people older than or equal to 60 years old). No interactions or significant differences were found for the variables ACE2, Ang II and Ang-(1–7)

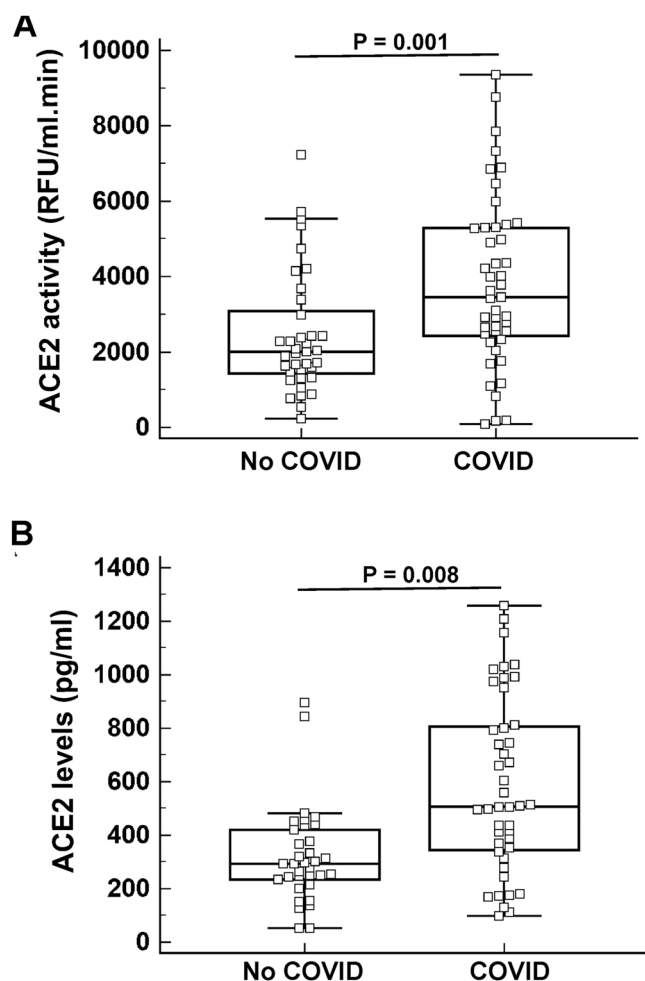


Fig. 2. sACE2 activity and protein levels in hospitalized COVID-19 patients excluding those under RAS blockade treatment: (A) sACE2 activity and (B) sACE2 protein levels in the plasma of healthy subjects (no COVID, $n = 33$) and hospitalized COVID-19 patients (COVID, $n = 66$), excluding those hypertensive hospitalized COVID-19 patients treated with RAS inhibitors. The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the boxes show the median values and the whiskers extending from both ends of the boxes are minimum and maximum values. Wilcoxon Test (Mann-Whitney U) test was applied for statistical analysis.

levels.

3. Results

3.1. Characteristics of the investigated population

Tables 1 and 2 show characteristics of the investigated population. Ninety-three adults (66 % men, 54 ± 16 years old), 58 % ($n = 54$) normotensive and 42 % ($n = 39$) hypertensive hospitalized with COVID-19 were investigated. Eighty-one (87 %) patients required respiratory support with simple face or reservoir mask during hospitalization and 12 (13 %) required intensive care unit admission with mechanical ventilation. While 92 % recovered from COVID-19, the remaining 8 % died. The most common comorbidities were diabetes (26 %), hypertension (44 %), obesity (5 %), ischemic cardiomyopathy (9 %), and chronic kidney disease (11 %). The median blood pressure of COVID-19 patients at hospitalization were 120 (IQR = 20) /80 (IQR = 10) mmHg and 136 (IQR = 30)/80 (IQR = 10) in normotensives and hypertensives, respectively. Sixty nine percent of those hypertensive COVID-19 patients were under ACEI/ARB treatment while the remaining (31 %) were treated with other antihypertensive medications.

Deidentified samples from healthy volunteer's outpatient adults (normotensive: $n = 33$, 48 % men, 51 ± 18 years old; and hypertensive: $n = 7$, 42 % men, 76.7 ± 6.6 years old) comprised the control group.

3.2. sACE2 activity and protein levels increased in COVID-19 patients

COVID-19 patients displayed significantly higher sACE2 enzymatic activity and protein levels compared to healthy subjects ($P = 0.001$ and $P < 0.001$, respectively; Fig. 1). We did not find differences in sACE2 enzymatic activity and protein levels between those COVID-19 patients hospitalized in general ward (required respiratory support with mask) versus those requiring intensive care unit admission (required respiratory support with mechanical ventilation). When we compared non-hypertensive healthy subjects with non-hypertensive COVID-19 patients we found that sACE2 enzymatic activity and protein levels were still higher in the COVID-19 group (Fig. 1).

When we compared the hypertensive population we found that hypertensive COVID-19 patients exhibited greater sACE2 levels compared to hypertensive healthy subjects (731.6 (IQR: 617.8) mg/mL in COVID-19 patients and 359.1 (IQR: 241.8) mg/mL in healthy subjects, $P = 0.03$, Mann Whitney U -test) while there was no difference in sACE2 activity between both groups (Fig. 1).

Within the COVID-19 group, sACE2 activity and protein levels were not different between normotensive and hypertensive-treated patients ($P = 0.485$) (Fig. 1). sACE2 activity was significantly higher in COVID-19 patients 60 years of age and older compared to patients younger than 60 years old (3621.4 (IQR: 3521.5) RFU/mL.min in COVID-19 patients and 5381.6 (IQR: 4551.6) RFU/mL.min in healthy subjects, $P = 0.05$), while sACE2 levels were not different (695.1 (IQR: 635.9) mg/mL in COVID-19 patients and 643.1 (IQR: 612.9) mg/mL in healthy subjects, $P = 0.485$).

Regarding the type of antihypertensive treatment, there was no difference in sACE2 activity and protein levels between hypertensive patients treated with ACEI/ARB ($n = 27$) and those treated with other antihypertensive medications ($n = 12$) ($P = 0.52$). We also did not find differences between those medicated with ACEI ($n = 23$) or ARB ($n = 4$) (data not shown).

When COVID-19 patients under ACEI/ARB treatment were excluded from analysis, sACE2 protein levels and activity remained significantly higher in COVID-19 patients than in healthy subjects ($P = 0.001$ and $P = 0.008$, respectively; Fig. 2).

3.3. Ang II and Ang-(1–7) levels decreased in COVID-19 patients

Ang II and Ang-(1–7) concentrations significantly decreased in COVID-19 patients compared to healthy controls (Ang II levels decreased from 56.4 [IQR = 59.2] to 40.0 [IQR = 39.7] pmol/L and Ang-[1–7] from 391.2 [IQR = 374.6] to 291.8 [IQR = 233.1] pmol/L, $P = 0.04$). Similar findings were observed when patients under ACEI/ARB treatment were excluded from analysis; Ang II and Ang-(1–7) levels remained significantly lower in COVID-19 patients compared to healthy subjects (Ang II levels decreased from 53.9 [IQR = 57.3] to 40.3 [IQR = 40.2] pmol/L and Ang-[1–7] from 421.8 [IQR = 397.4] to 277.0 [IQR = 245.5] pmol/L ($P = 0.04$ and 0.05, respectively; Fig. 3). In addition, median Ang II levels were significantly higher in men than women with COVID-19 (55.6 [IQR = 52.5] pmol/L in men versus 36.2 [IQR = 24.8] pmol/L in women, $P = 0.03$), while there was no difference by sex in Ang-(1–7) levels.

When we compared non-hypertensive healthy subjects with non-hypertensive COVID-19 patients we found that Ang-(1–7) levels significantly decreased ($P = 0.01$) while Ang II did not change in the COVID-19 group (Fig. 3C and D).

The ratio Ang-(1–7)/Ang II was not different between healthy and COVID-19 patients, suggesting no difference in the rate of Ang II conversion into Ang-(1–7) (Fig. 4E). No difference in the ratio Ang-(1–7)/Ang II was found between normotensive and hypertensive COVID-19

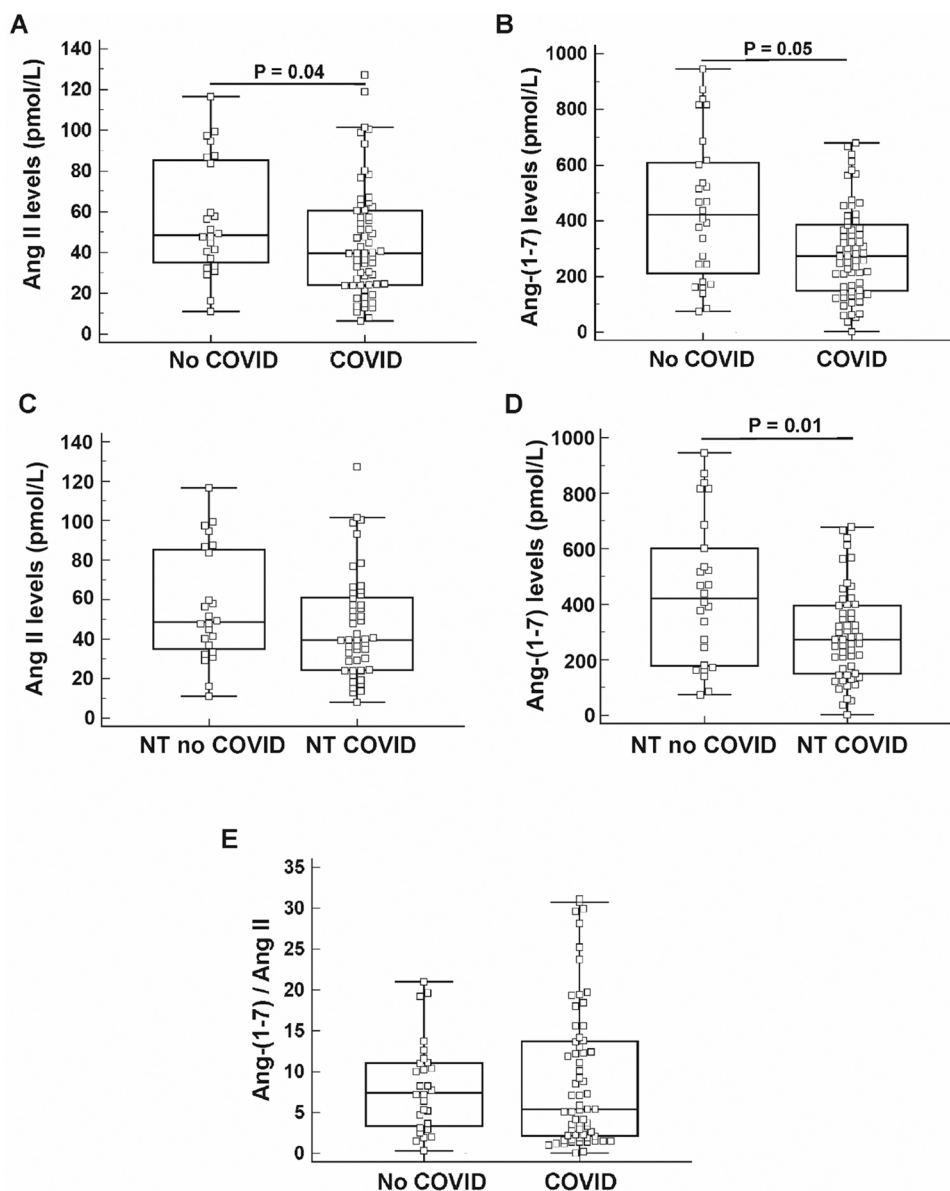


Fig. 3. Ang II and Ang-(1-7) levels and Ang-(1-7)/Ang II ratio in hospitalized COVID-19 patients. (A) Ang II and (B) Ang-(1-7) levels and (E) Ang-(1-7)/Ang II ratio in the plasma of healthy subjects (no COVID, $n = 33$) and hospitalized COVID-19 patients (COVID, $n = 66$), excluding those hospitalized COVID-19 patients treated with RAS inhibitors. (C) Ang II and (D) Ang-(1-7) levels in the plasma of non-hypertensive healthy subjects (NT no COVID, $n = 33$) and non-hypertensive hospitalized COVID-19 patients (NT COVID, $n = 54$). The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the boxes show the median values and the whiskers extending from both ends of the boxes are minimum and maximum values. Wilcoxon test (Mann-Whitney U -test) was applied for statistical analysis.

patients (normotensive: 5.35 (IQR: 10.80)) and hypertensive: 10.70 (IQR: 16.25).

3.4. COVID-19 patients exhibited increased IL-6 and IL-8 levels

Additionally, due to the relationship between RAS and inflammation [1,17,18], we evaluated IL levels. COVID-19 patients exhibited higher levels of IL-6 and IL-8 than healthy subjects though no difference in IL-1 β and IL-10 (Fig. 4). No difference in IL levels was detected between normotensive and hypertensive COVID-19 patients (data not shown).

There was no correlation between sACE2 activity and protein levels with IL-6 and IL-8 levels. However, we found a negative correlation between sACE2 activity and IL-6 in COVID-19 patients who were 60 years of age and older ($r = -0.44$ (CI -0.75 to -0.15); $P = 0.02$) and between sACE2 activity and IL-8 in hypertensive COVID-19 patients ($r = -0.39$ (CI -0.69 to -0.11); $P = 0.02$).

4. Discussion

In this study we report a decrease in plasmatic levels of Ang II and Ang-(1-7) and an increase in sACE2 activity and protein levels in

COVID-19 patients. In agreement, a decrease in Ang-(1-7) levels with similar plasmatic concentrations as those observed in our study has been reported in COVID-19 patients [19]. The finding that Ang-(1-7) was reduced in COVID-19 patients seemed not to correlate with the increase in sACE2 activity (present results), which should reduce Ang II but increase Ang-(1-7). However, some reports have shown an increase in ACE2 in plasma of COVID-19 patients without a concomitant decrease in Ang II and an increase in Ang-(1-7) levels. For instance, ACE2 was shown to be increased in COVID-19 patients together with an increased Ang II levels which subsequently decreased after 9–11 days despite the fact that ACE2 was still high [6]; however, others reported no change [10,11,20–22] or a decrease [23–25] in Ang II levels in COVID-19 patients. The reduction in Ang II may be related to the decrease in Ang I or ACE activity reported in COVID-19 patients [19,26].

Regarding Ang-(1-7) levels in COVID-19 patients, some reports have shown an increase [6,10,27] while others a decrease or no change [11,19,28]. Thus, despite the fact that COVID-19 is associated with an increased ACE2 [6–9,16,29], conflicting results were reported regarding Ang II and Ang-(1-7) levels [30]. Our work showed that even sACE2 activity was increased, the ratio Ang-(1-7)/Ang II was not changed in the plasma of COVID-19 patients, reflecting that the rate of conversion

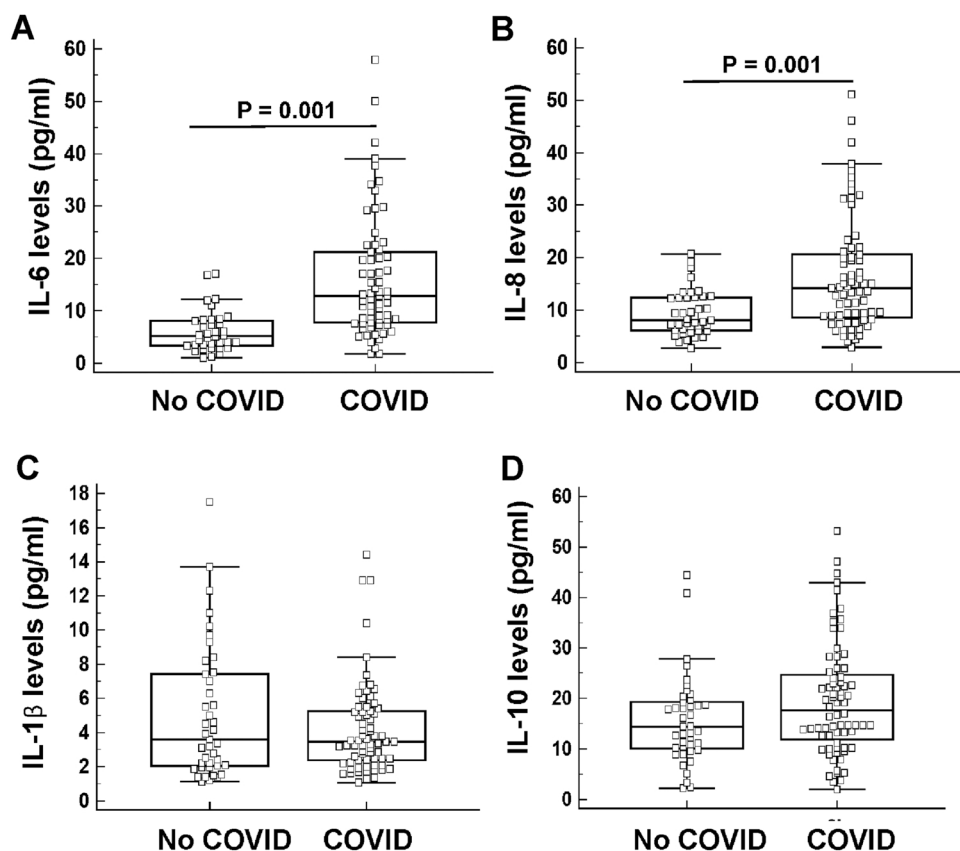


Fig. 4. Plasmatic ILs levels in hospitalized COVID-19 patients: (A) IL-6, (B) IL-8, (C) IL-1 β , and (D) IL-10 levels in the plasma of healthy subjects (no COVID, $n = 33$) and hospitalized COVID-19 patients (COVID, $n = 93$). The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The bands within the boxes show the median values and the whiskers extending from both ends of the boxes are minimum and maximum values. Wilcoxon Test (Mann-Whitney U) test was applied for statistical analysis.

of Ang II into Ang-(1–7) was not modified. This result suggests that despite the fact that sACE2 increased in COVID-19 patients, sACE2 seems not to be involved in Ang II conversion to Ang-(1–7). In fact, Ang II is not the only substrate of ACE2 since others circulating compounds may be metabolized by ACE2 [31]. On the other hand, others enzymes may be involved in plasmatic Ang II metabolism such as prolyl oligopeptidase [32]. Furthermore, Ang-(1–7) can be formed not only from Ang II but also from Ang I. Neutral endopeptidase (EC 3.4.24.11, EP 24.11), neprilysin, thimet oligopeptidase (EC 3.4.24.15) and prolyl oligopeptidase (EC 3.4.21.26) cleave the bond at residues Pro⁷-Phe⁸ of Ang I, thus generating Ang-(1–7) [30,33]. In fact, COVID-19 is associated with low circulating plasma levels of Ang I and Ang-(1–7) [19]. Thus, different metabolic pathways and not only ACE2 may contribute to changes in circulating Ang II and Ang-(1–7) levels [34].

In agreement with previous studies [6–9,16,27] we observed that COVID-19 patients exhibited greater sACE2 protein levels and enzymatic activity compared to healthy subjects. The increased sACE2 may reflect higher ACE2 shedding. ACE2 shedding is mainly driven by ADAM17 [2,3]. In accord, severe COVID-19 patients have been shown to exhibit an increase in plasmatic ADAM17 [35]. Inhibition of ADAM17 by a sheddase inhibitor or by a specific siRNA potently suppressed SARS-CoV-2 infection [36]. To our knowledge, there is no report on ADAM17 expression in the membrane of COVID-19 patients to confirm that the increased sACE2 is correlated with ADAM17 levels. Wang et al. [27] investigated the prognostic value of sACE2 and TNF receptors (sTNFRs), another target of ADAM17, at baseline and during repeat sampling as surrogate markers of ADAM17 activity. They found that baseline sACE2 and sTNFRs were elevated in COVID-19 patients compared to healthy controls. At repeat sampling, the temporal profile of sACE2 and sTNFRs showed a substantial increase in biomarkers related to ADAM17 activity [27]. On the other hand, ACE2 shedding modulates SARS-CoV-2 infectivity through a mechanism involving the Ang type 1 receptor (AT1R). SARS-CoV-2 exploits receptor-mediated

endocytosis through interaction between its spike with sACE2 via AT1R or vasopressin receptor [36,37]. Thus, COVID-19 patients would have augmented sACE2 due to an increased ADAM17 activity, but at the same time SARS-CoV2 interacts with sACE2 to enter into the cells through an AT1R- or vasopressin receptor-mediated mechanism [36, 37]. However, sACE2 shedding results not only from ADAM17 but also from SARS-CoV-2 infection. It has been reported that SARS-CoV-2 infection induces shedding of ACE2 from cell membranes, leading to increased levels of the soluble active form of ACE2 in situ and in plasma of infected patients [16].

Our study showed no difference in sACE2 activity or protein levels between normotensive and hypertensive-treated COVID-19 patients, even if those hypertensive patients were treated with ACEI/ARB or other antihypertensive treatment. In accord, in a larger cohort of COVID-19 patients ($n = 218$) it was shown that there was no significant difference in sACE2 levels between normotensive and hypertensive treated subjects, not even when the type of antihypertensive treatment was analyzed [22]. In agreement, sACE2 levels during COVID-19 did not differ depending on the presence of risk factors for severe COVID-19 infection (with the exception of male sex) and were not affected by RAS inhibition [29]. Accordingly, we have recently reported that ACEI/ARB treatment did not modify ACE2 protein expression in type II pneumocytes [12] which are key cells for lung homeostasis. In contrast, a report investigating ACE2 activity in plasma of hypertensive COVID-19 patients who were not experiencing severe COVID-19, found an increase in sACE2 activity under ACEI treatment compared to non-medicated COVID-19 people though with a smaller sample size ($n = 9–10$) [10].

In agreement with previous studies [38–40], we found that COVID-19 patients displayed increased proinflammatory cytokines such as IL-6 and IL-8. Recently, it has been shown that the increased in ACE2 function in plasma of SARS-CoV-2 infected patients correlated with viral load and IL-6 levels [16]. In contrast, we found that the augmented

sACE2 protein levels in our COVID-19 patients did not correlate with the increase in IL-6 and IL-8. This lack of correlation may be related to the reported over expression of ADAM17 described in COVID-19 patients [35] suggesting that ACE2 seems to be a marker of infection without any relationship with the severity of the disease. In accord, sACE2 activity and protein levels did not correlate with the severity of disease (present results), which may be due to the small sample size with severe or fatal outcome. In accord, it has been shown lack of association between sACE2 protein levels and severity of COVID-19 [27,29]. In contrast, through repetitive measurement of sACE2 in patients with severe COVID-19 it was shown that the early stage of aberrant ACE2 shedding reflects the infectious stage of the illness, while persistent elevation in sACE2 results in progressive end-organ injury due to loss of tissue ACE2 and correlates with mortality [27]. We observed an inverse correlation between sACE2 activity and IL-6 in COVID-19 patients who were 60 years of age and older. In accord with our results, it has been shown that sACE2 did not display positive correlations with IL6 in COVID-19 patients [29]. According to a recent report, sACE2 and sACE levels had different correlations with markers of inflammation and endothelial dysfunction, which may imply an association with different types of cell injury or release from different cell types or vascular beds [29].

Our study is limited by the sample size and heterogeneity on duration of illness; however, our results support the involvement of the RAS in COVID-19, even when patients under ACEI/ARB treatment were excluded. Another limitation was the low number of samples from hypertensive healthy patients.

5. Conclusions

In summary, our study revealed that hospitalized COVID-19 patients exhibited decreased circulating Ang-(1–7) and Ang II levels and increased sACE2 enzymatic activity and protein levels, with no difference between normotensive and hypertensive treated COVID-19 patients. The increased circulating sACE2 levels may reflect increased ACE2 expression, enhanced ACE2 shedding, or both and suggest higher basal ACE2 expression in these patients' plasma membranes and therefore greater susceptibility to infection. Unfortunately we do not have access to lungs of COVID-19 patients to test this hypothesis.

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CRediT authorship contribution statement

Mauro Silva: Data curation, Methodology, Investigation, Validation, Writing – review & editing. **Gerardo Corradi:** Data curation, Methodology, Investigation. **Juan Perez Duhalde:** Data curation, Investigation. **Myriam Nuñez:** statistical formal analysis. **Eliana Cela:** Data curation, Methodology. **Daniel Gonzales Maglio:** Methodology, Investigation. **Ana Brizzio:** Methodology. **Martin Salazar:** Formal analysis, writing. **Walter Espeche:** Formal analysis, Writing – review & editing, Visualization. **Mariela Gironacci:** Conceptualization, Methodology, Supervision, Project administration, Visualization, Funding acquisition, Writing – original draft.

Conflict of interest statement

All authors have participated in the work and have reviewed and agree with the content of the article. None of the article contents are under consideration for publication in any other journal or have been published in any journal. The authors declare that there are no conflicts of interest.

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