Sustained Dysregulation of the Plasma Renin-angiotensin System in Acute COVID-19

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Abstract

SARS-CoV-2 enters cells by binding to angiotensin-converting enzyme 2 (ACE2), and COVID-19 infection may therefore induce changes in the renin-angiotensin system (RAS). To determine the effects of COVID-19 on plasma RAS components, we measured plasma ACE, ACE2, and angiotensins I, (1-7), and II in 46 adults with COVID-19 at hospital admission and on days 2, 4, 7 and 14, compared to 50 blood donors (controls). We compared survivors vs. non-survivors, males vs. females, ventilated vs. not ventilated, and angiotensin receptor blocker (ARB) and angiotensin-converting enzyme (ACE) inhibitor-exposed vs. not exposed. At admission, COVID-19 patients had higher plasma levels of ACE ($p=0.012$), ACE2 ($p=0.001$) and angiotensin-(1-7) ($p<0.001$) than controls. Plasma ACE and ACE2 remained elevated for 14 days in COVID-19 patients, while plasma angiotensin-(1-7) decreased after 7 days. In adjusted analyses, plasma ACE was higher in males vs. females ($p=0.042$), and plasma angiotensin I was significantly lower in ventilated vs. non-ventilated patients ($p=0.001$). In summary, plasma ACE and ACE2 are increased for at least 14 days in patients with COVID-19 infection. Angiotensin-(1-7) levels are also elevated, but decline after 7 days. The results indicate dysregulation of the RAS with COVID-19, with increased circulating ACE2 throughout the course of infection.

Clinical Trial Registration: <https://clinicaltrials.gov/> Unique Identifier: NCT04510623

Introduction

The COVID-19 pandemic continues to grow exponentially with more than 60 million confirmed cases and 1.5 million deaths worldwide. There are no effective antiviral treatments, although dexamethasone, a host modulating anti-inflammatory intervention, lowers mortality of severe COVID-19 [1]. The World Health Organization (WHO) and others are launching clinical trials of novel anti-virals [2] and other host response modulators (anti-inflammatory agents [3] and immunomodulatory therapies [4]). Notably, the critical illness complications of COVID-19, such as septic shock, acute respiratory distress syndrome (ARDS), and acute kidney injury (AKI) [5, 6, 7] could be caused in part by the host response. This may be especially pertinent to the renin-angiotensin system (RAS) because of the interactions of SARS-CoV-2, the etiologic agent of COVID-19, with angiotensin-converting enzyme 2 (ACE2), the plasma membrane protein that mediates its cellular entry [8, 9].

As a mono-carboxypeptidase, ACE2 cleaves one amino acid from the C-terminal of the decapeptide angiotensin I to yield angiotensin-(1–9), and similarly potently converts the octapeptide angiotensin II to angiotensin-(1–7). While angiotensin II is a vasoconstrictor with inflammatory, pro-coagulant and endothelial permeability-inducing actions, its degradative product angiotensin-(1–7) is an endogenous vasodilator with counter-regulatory, anti-inflammatory properties [10]. ACE2 down-regulation or inhibition increases tissue angiotensin II levels, and decreases protective angiotensin-(1–7), which could contribute to enhanced organ dysfunction and mortality.

The SARS-CoV-2 spike protein binds with high affinity to ACE2, facilitating its entry into cells [8, 9], and this interaction could reduce ACE2 expression and activity. In COVID-19 infection this effect may exacerbate lung, cardiac, or other organ injury by increasing inflammation, coagulation and endothelial permeability. ACE2 is downregulated in H1N1, H5N1, H7N9, and SARS, and angiotensin II worsens lung injury in influenza models [11, 12, 13]. Angiotensin II levels increase in human influenza H5N1 compared to healthy controls, increase during the course of H5N1, and are associated with viral load, disease progression and mortality [12]. Similarly, plasma angiotensin II levels are associated with severity and mortality of H7N9 [13]. The effect of COVID-19 on plasma angiotensin II has not been extensively studied, although levels were reported to be increased in a small cohort of adult patients [14]. These findings provide a rationale for use of angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEi) in such infections, and indeed current evidence suggests lack of harm with ongoing use [15].

The RAS (and ACE2 in particular) is likely disrupted in a complex fashion in COVID-19 and could cause some of the adverse clinical phenotypes. However, no studies have evaluated the relationships of plasma ACE, ACE2, and angiotensins I, (1–7) and II in COVID-19 patients compared to healthy controls. Our primary objective was to determine if plasma RAS component

levels in COVID-19 differ from control subjects. The secondary aim was to study potential differences in RAS components in COVID-19 between males and females, survivors and non-survivors, ventilated vs. not ventilated patients, and those exposed vs. not exposed to ARBs and ACEi.

Results

COVID-19 Patients vs. Controls

The baseline characteristics of hospitalized COVID-19 patients (n = 46) are shown in Table 1, and were similar to other reports [7]. Patients were 65 years (mean), 72% male, 52% with comorbidities, 44% admitted to ICU, 35% ventilated, 33% requiring vasopressors, and mortality rate at 28 days was 21%. ARB or ACEi use was found in 8.7% (n = 4) and 17% (n = 8) respectively prior to admission and was continued in hospital in 6.8% (n = 3) and 11.1% (n = 5) respectively. Controls (n = 50) consisted of 25 males and 25 female blood donors with mean age 40 years.

Table 1
Baseline characteristics, mortality, use of invasive ventilation, and use of ARB and ACE inhibitors in patients with COVID-19 and healthy controls

	28-day mortality			Invasive mechanical ventilation		Sex		ARBs/ACEi‡		
Variable	COVID-19 (n = 46)	No (n = 38)	Yes (n = 8)	No (n = 30)	Yes (n = 16)	Male (n = 33)	Female (n = 13)	No ARBs/ACEi (n = 34)	ARBs (n = 4)	ACEi (n = 8)
Age										
Mean (SD)	65.2 (16.7)	61.2 (15.0)	84.4 (9.5)	65.0 (19.1)	65.6 (11.3)	66.3 (15.3)	62.5 (20.3)	62.3 (17.4)	64.8 (7.4)	77.8 (10.8)
Median (IQR)	64.5 (52.0, 79.0)	58.5 (49.0, 72.0)	87.0 (79.0, 91.5)	68.5 (48.0, 81.0)	63.0 (58.0, 73.5)	63.0 (55.0, 79.0)	68.0 (42.0, 80.0)	58.5 (48.0, 76.0)	66.5 (59.0, 70.5)	79.5 (75.0, 84.0)
Range	(34.0, 94.0)	(34.0, 90.0)	(66.0, 94.0)	(34.0, 94.0)	(48.0, 90.0)	(40.0, 94.0)	(34.0, 90.0)	(34.0, 94.0)	(55.0, 71.0)	(55.0, 90.0)
Sex, n (%)										
Male	33 (71.7)	27 (71.1)	6 (75.0)	19 (63.3)	14 (87.5)	33 (100.0)	0 (0.0)	25 (73.5)	3 (75.0)	5 (62.5)
Female	13 (28.3)	11 (28.9)	2 (25.0)	11 (36.7)	2 (12.5)	0 (0.0)	13 (100.0)	9 (26.5)	1 (25.0)	3 (37.5)
Co-morbidities, n (%) ^										
Any of the following	24 (52.2)	18 (47.4)	6 (75.0)	17 (56.7)	7 (43.8)	18 (54.5)	6 (46.2)	12 (35.3)	4 (100.0)	8 (100.0)
Chronic cardiac disease	10 (21.7)	7 (18.4)	3 (37.5)	9 (30.0)	1 (6.3)	7 (21.2)	3 (23.1)	6 (17.6)	0 (0.0)	4 (50.0)
Chronic kidney disease	5 (10.9)	2 (5.3)	3 (37.5)	3 (10.0)	2 (12.5)	4 (12.1)	1 (7.7)	3 (8.8)	0 (0.0)	2 (25.0)
Diabetes	13 (28.3)	9 (23.7)	4 (50.0)	8 (26.7)	5 (31.3)	12 (36.4)	1 (7.7)	7 (20.6)	3 (75.0)	3 (37.5)
With complications	3 (6.5)	1 (2.6)	2 (25.0)	2 (6.7)	1 (6.3)	3 (9.1)	0 (0.0)	3 (8.8)	0 (0.0)	0 (0.0)
Without complications	10 (21.7)	8 (21.1)	2 (25.0)	6 (20.0)	4 (25.0)	9 (27.3)	1 (7.7)	4 (11.8)	3 (75.0)	3 (37.5)
Hypertension	20 (43.5)	14 (36.8)	6 (75.0)	13 (43.3)	7 (43.8)	15 (45.5)	5 (38.5)	8 (23.5)	4 (100.0)	8 (100.0)
ARBs prior to admission, n (%)	4 (8.7)	4 (10.5)	0 (0.0)	1 (3.3)	3 (18.8)	3 (9.1)	1 (7.7)	0 (0.0)	4 (100.0)	0 (0.0)
ACE inhibitors prior to admission, n (%)	8 (17.4)	6 (15.8)	2 (25.0)	7 (23.3)	1 (6.3)	5 (15.2)	3 (23.1)	0 (0.0)	0 (0.0)	8 (100.0)

*Multiple categories can be selected for each patient; ‡ Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; ICU, intensive care unit, RRT, renal replacement therapy.

	28-day mortality			Invasive mechanical ventilation		Sex		ARBs/ACEi‡		
Admitted to ICU, n (%)	20 (43.5)	15 (39.5)	5 (62.5)	4 (13.3)	16 (100.0)	16 (48.5)	4 (30.8)	15 (44.1)	3 (75.0)	2 (25.0)
Treatment during hospitalization										
ARBs, n (%)	3/44 (6.8)	3/36 (8.3)	0/8 (0.0)	2/29 (6.9)	1/15 (6.7)	2/33 (6.1)	1/11 (9.1)	1/33 (3.0)	2/4 (50.0)	0/7 (0.0)
ACEi, n (%)	5/45 (11.1)	4/37 (10.8)	1/8 (12.5)	5/30 (16.7)	0/15 (0.0)	2/33 (6.1)	3/12 (25.0)	1/33 (3.0)	0/4 (0.0)	4/8 (50.0)
Mechanical ventilation, n (%)	16/46 (34.8)	12/38 (31.6)	4/8 (50.0)	0/30 (0.0)	16/16 (100.0)	14/33 (42.4)	2/13 (15.4)	12/34 (35.3)	3/4 (75.0)	1/8 (12.5)
RRT, n (%)	4/41 (9.8)	2/34 (5.9)	2/7 (28.6)	0/29 (0.0)	4/12 (33.3)	4/29 (13.8)	0/12 (0.0)	3/29 (10.3)	1/4 (25.0)	0/8 (0.0)
Vasopressors, n (%)	15/45 (33.3)	10/37 (27.0)	5/8 (62.5)	2/30 (6.7)	13/15 (86.7)	13/33 (39.4)	2/12 (16.7)	13/33 (39.4)	1/4 (25.0)	1/8 (12.5)
Antiviral agent, n (%)	8/45 (17.8)	6/37 (16.2)	2/8 (25.0)	4/30 (13.3)	4/15 (26.7)	6/33 (18.2)	2/12 (16.7)	7/33 (21.2)	0/4 (0.0)	1/8 (12.5)
Patient in-hospital outcome, n (%)										
Still in hospital	1 (2.2)	1 (2.6)	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	1 (7.7)	1 (2.9)	0 (0.0)	0 (0.0)
Death	10 (21.7)	3 (7.9)	7 (87.5)	3 (10.0)	7 (43.8)	9 (27.3)	1 (7.7)	9 (26.5)	0 (0.0)	1 (12.5)
Discharged alive	28 (60.9)	27 (71.1)	1 (12.5)	24 (80.0)	4 (25.0)	17 (51.5)	11 (84.6)	20 (58.8)	2 (50.0)	6 (75.0)
Discharged to other facility	7 (15.2)	7 (18.4)	0 (0.0)	3 (10.0)	4 (25.0)	7 (21.2)	0 (0.0)	4 (11.8)	2 (50.0)	1 (12.5)
Hospital length of stay (among those who discharged alive) (days)										
Median (IQR)	7.0 (3.5, 14.0)	7.0 (3.0, 14.0)	8.0 (8.0, 8.0)	7.0 (3.0, 10.5)	17.5 (13.0, 28.5)	7.0 (3.0, 11.0)	8.0 (4.0, 17.0)	7.0 (3.0, 14.0)	21.5 (4.0, 39.0)	7.5 (7.0, 11.0)
Mean (SD)	10.5 (10.8)	10.6 (11.0)	8.0 (.)	8.8 (9.7)	20.8 (12.8)	9.5 (9.2)	12.0 (13.4)	8.5 (6.0)	21.5 (24.7)	13.5 (17.3)
Range	(0.0, 48.0)	(0.0, 48.0)	(8.0, 8.0)	(0.0, 48.0)	(9.0, 39.0)	(0.0, 39.0)	(0.0, 48.0)	(0.0, 19.0)	(4.0, 39.0)	(0.0, 48.0)
Discharged from ICU, n (%)										

*Multiple categories can be selected for each patient; ‡ Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; ICU, intensive care unit, RRT, renal replacement therapy.

	28-day mortality			Invasive mechanical ventilation		Sex		ARBs/ACEi‡		
Still in ICU	1 (5.0)	1 (6.7)	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	1 (25.0)	1 (6.7)	0 (0.0)	0 (0.0)
Death in ICU	6 (30.0)	2 (13.3)	4 (80.0)	1 (25.0)	5 (31.3)	6 (37.5)	0 (0.0)	5 (33.3)	0 (0.0)	1 (50.0)
Discharged alive from ICU	13 (65.0)	12 (80.0)	1 (20.0)	3 (75.0)	10 (62.5)	10 (62.5)	3 (75.0)	9 (60.0)	3 (100.0)	1 (50.0)
ICU length of stay (among those who discharged from ICU alive) (days)										
Median (IQR)	9.5 (5.5, 23.0)	10.0 (5.0, 29.0)	9.0 (9.0, 9.0)	3.0 (1.0, 8.0)	12.0 (9.0, 29.0)	12.0 (6.0, 29.0)	8.0 (1.0, 9.0)	8.5 (5.5, 13.5)	29.0 (12.0, 43.0)	3.0 (3.0, 3.0)
Mean (SD)	14.6 (13.2)	15.1 (13.7)	9.0 (.)	4.0 (3.6)	18.1 (13.4)	17.4 (14.0)	6.0 (4.4)	11.0 (9.7)	28.0 (15.5)	3.0 (.)
Range	(1.0, 43.0)	(1.0, 43.0)	(9.0, 9.0)	(1.0, 8.0)	(5.0, 43.0)	(3.0, 43.0)	(1.0, 9.0)	(1.0, 32.0)	(12.0, 43.0)	(3.0, 3.0)
Time to death (among those who died) (days)										
Median (IQR)	17.0 (13.0, 36.0)	37.0 (36.0, 62.0)	13.0 (4.0, 20.0)	4.0 (3.0, 14.0)	24.0 (13.0, 37.0)	20.0 (13.0, 36.0)	13.0 (13.0, 13.0)	20.0 (13.0, 36.0)	-	13.0 (13.0, 13.0)
Mean (SD)	22.6 (18.1)	45.0 (14.7)	13.0 (7.7)	7.0 (6.1)	29.3 (17.4)	23.7 (18.8)	13.0 (.)	23.7 (18.8)	-	13.0 (.)
Range	(3.0, 62.0)	(36.0, 62.0)	(3.0, 24.0)	(3.0, 14.0)	(13.0, 62.0)	(3.0, 62.0)	(13.0, 13.0)	(3.0, 62.0)	-	(13.0, 13.0)
*Multiple categories can be selected for each patient; ‡ Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin-converting enzyme inhibitor; ICU, intensive care unit, RRT, renal replacement therapy.										

At baseline, significantly higher plasma levels of ACE were observed in COVID-19 patients (median 47.5 U/ml) than controls (median 35.1 U/ml, $p = 0.012$). Plasma ACE2 (1.86 ng/ml vs. 0.51 ng/ml, $p = 0.001$) and angiotensin-(1–7) (8.39 vs. 0.98 pg/ml, $p < 0.001$) were also elevated compared to controls (Fig. 1; Supplementary Table 1). There were no differences between COVID-19 patients and controls in plasma angiotensin I (40.0 pg/ml vs. 37.4 pg/ml, $p = 0.96$) or plasma angiotensin II (97.2 pg/ml vs. 103.6 pg/ml, $p = 0.41$).

No significant correlations were observed between any pairs of RAS components in COVID-19 patients ($\rho \leq 0.36$) and controls ($\rho \leq 0.27$; Supplementary Table 2).

Time Course of RAS Levels in COVID-19

Plasma ACE and ACE2 remained elevated for 14 days after admission, while plasma angiotensin-(1–7) levels were high until days 7 and 14, when significant decreases were noted. There were no significant changes in plasma angiotensins I or II from baseline or on days 2, 4, 7 and 14 after hospital admission (Fig. 2; Supplementary Table 3).

COVID-19 and Sex Differences in RAS Components

Plasma angiotensin-(1–7) levels were higher in males than females ($p = 0.027$, Supplementary Table 1), but this increase was not significant after adjustment for confounders (age, chronic cardiac disease, chronic kidney disease, diabetes and hypertension). However, plasma ACE was significantly higher in males than females with acute COVID-19, after adjusting for confounders (higher by 9.77 pg/ml, (95% CI: 0.39, 19.14, $p = 0.042$ (Table 2, Supplementary Table 1).

Table 2
*RAS components in COVID-19 – Adjusted Analyses

Estimated difference in median protein level										
	Death vs. survived		Ventilated vs. not		Male vs Female		ACEi vs. no ARBs/ACEi		ACEi vs. no ACEi	
	Diff (95% CI)	†P	Diff (95% CI)	P	Diff (95% CI)	P	Diff (95% CI)	P	Diff (95% CI)	P
ACE (U/ml)	-0.26 (-12.27, 11.75)	0.966	1.14 (-7.19, 9.48)	0.783	9.77 (0.39, 19.14)	0.042	-17.51 (-39.89, 4.87)	0.121	-14.85 (-33.41, 3.71)	0.114
ACE2 (ng/ml)	-0.19 (-18.83, 18.46)	0.984	0.10 (-20.18, 20.39)	0.992	-4.35 (-29.39, 20.70)	0.727	-1.58 (-39.42, 36.25)	0.933	-2.19 (-41.59, 37.21)	0.911
Angiotensin I (pg/ml)	1.46 (-12.47, 15.39)	0.833	-11.58 (-18.35, -4.82)	0.001	12.41 (-0.05, 24.87)	0.051	7.05 (-10.73, 24.83)	0.426	14.48 (-5.01, 33.96)	0.141
Angiotensin 1–7 (pg/ml)	13.40 (-16.52, 43.32)	0.370	5.11 (-7.59, 17.81)	0.421	5.01 (-7.55, 17.57)	0.425	-5.50 (-36.22, 25.21)	0.718	-1.75 (-27.65, 24.15)	0.892
Angiotensin II (pg/ml)	33.17 (-32.89, 99.24)	0.315	23.31 (-28.46, 75.08)	0.367	-46.86 (-140.85, 47.13)	0.319	-65.68 (-191.65, 60.29)	0.296	-42.56 (-143.18, 58.05)	0.397

*Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2, †P value was based on quantile regression adjusted for age, sex, chronic cardiac disease, chronic kidney disease, diabetes and hypertension. Adjusted comparisons cannot be performed for the ARBs group due to low number of patients ($n = 4$). For the “No ACEi” group, 4 patients have used ARBs.

COVID-19, Mortality and Invasive Ventilation

In unadjusted analysis, there were no significant differences in any plasma RAS enzymes or peptides between COVID-19 patients who died or survived, and between patients who did or did not require invasive ventilation (Fig. 1; Supplementary Table 1). However, in adjusted analyses, plasma angiotensin I was significantly lower in ventilated vs. non-ventilated patients (lower by 11.6 pg/ml, (95% CI: 4.8, 18.4, $p = 0.001$), Table 2).

ARBs and ACEi in COVID-19

In unadjusted analysis there were significantly lower plasma levels of ACE2 in patients who were on ACEi compared to those on ARBs, and lower angiotensin II levels in patients on ACEi compared to ACEi-unexposed patients (Fig. 1; Supplementary Table 1). However, these differences, as well as the differences in other RAS components, were not significant after adjustment for confounders (Table 2). Plasma ACE2 was also significantly lower in ACEi-exposed vs. ARB-exposed patients (unadjusted: 0.70 vs. 4.78 ng/ml, $p = 0.011$) (Fig. 1; Supplementary Table 1). There were no significant differences between ARB/ACEi-exposed vs. not exposed in other RAS components after confounder adjustment.

Discussion

In this study of circulating RAS components in COVID-19 patients, we observed significantly higher plasma levels of RAS components (ACE, ACE2 and angiotensin-(1–7)) than controls. The RAS pathway was severely dysregulated after

hospitalization for acute COVID-19 with persistent elevations in plasma ACE and ACE2 over the first 14 days of hospitalization, while a decrease in plasma angiotensin-(1–7) occurred only after 7 days. In unadjusted analysis, plasma ACE2 was significantly lower in patients who were on ACEi compared to those on ARBs. ACEi use was also associated with lower plasma angiotensin II levels compared to patients not on ACEi, and angiotensin II was significantly lower in ACEi-exposed vs. ARB-exposed patients. Finally, in adjusted analysis, plasma ACE was significantly higher in males than females, and plasma angiotensin I was significantly lower in ventilated vs. non-ventilated patients.

To our knowledge, this is the first report of a panel of plasma RAS components that included ACE, ACE2 and angiotensins I, (I-7) and II, comparing patients hospitalized with COVID-19 to controls. We adjusted analyses for age and co-morbidities since these are associated with mortality with COVID-19 and because ACE2 is altered by diabetes and kidney disease [16, 17]. Our results differ from Kintscher et al. [18], who studied COVID-19-positive patients visiting the emergency room and found no differences in plasma ACE2 activity or angiotensin peptides compared to healthy controls. Rieder et al. [19] also found no difference in plasma ACE2 between COVID 19-positive and COVID-19-negative emergency department patients. We postulate that COVID-19 may modulate RAS components according to stage and severity of infection. At early stages of COVID-19, RAS components may not differ from healthy controls, while later in the course or with severe infection requiring hospitalization, RAS dysregulation becomes evident, as shown here. We also demonstrated that plasma ACE and ACE2 remain increased for at least 14 days after hospitalization. In a non-peer reviewed preprint, Patel et al. found that plasma ACE2 activity remained elevated for up to 114 days after onset of acute COVID-19 [20]. These data therefore suggest that in clinical trials of ARB, ACEi or recombinant human ACE2 use in COVID-19, the intervention should be maintained for at least 14 days.

ACE is located on the luminal surface of endothelial cells, modulates innate and adaptive immunity [21], and is increased in lung inflammatory disease. In the present study, plasma ACE may have increased in COVID-19 patients due to direct SARS-CoV-2 injury, or indirectly by inflammation and/or acute lung injury causing release of ACE into the circulation as soluble enzyme. In non-COVID-19 acute respiratory distress syndrome (ARDS), loss of cell-associated ACE activity by this mechanism has been postulated to lead to accumulation of plasma angiotensin I, which correlates with non-survival in these patients [22].

COVID-19 was also associated with increased plasma ACE2 compared to controls in our study. Circulating soluble ACE2 may have increased conversion of angiotensin II to angiotensin-(1–7), consistent with our finding that angiotensin-(1–7) levels were higher in COVID-19 patients. About 97–98% of ACE2 is membrane-bound, and can be released by a disintegrin and metalloproteinase (ADAM)-17-mediated shedding, thereby increasing the normal 2–3% of ACE2 that is circulating as soluble ACE2 [23]. Such shedding may itself be stimulated by local angiotensin II [24], or by inflammatory mediators such as interleukin (IL)-1 β or tumor necrosis factor (TNF)- α [25]. Accordingly, we suggest that SARS-Cov-2-induced cell injury led to release of membrane-bound ACE2, thereby increasing plasma ACE2 (Fig. 3).

ACE2 expression is widespread in lung, heart, kidney, small intestine and endothelial cells [26, 27]. Of note, circulating ACE2 may be protective in acute viral infection: higher circulating levels of ACE2 were associated with better outcomes of influenza A (H7N9) [28] and recombinant human ACE2 improves oxygen indices in acute respiratory distress syndrome [29]. Plasma ACE2 is also increased in patients with heart failure, more so in males than females [30]. On the other hand, increased plasma ACE2 has recently been identified as the most predictive biomarker of increased mortality in a large (n = 10,753) cardiovascular cohort study [31]. Further studies are required to uncover the pathophysiologic significance of circulating ACE2 in diverse disease states.

Plasma angiotensin II levels were not different between COVID-19 patients and controls in our study. Similarly, Rieder et al. [19] compared COVID-19 positive patients to COVID-19-negative patients in the emergency department and found no difference in plasma angiotensin II. In smaller cohorts, plasma angiotensin II levels were reported as increased in COVID-19 patients compared to healthy controls [14] and higher in critically ill than non-critically ill COVID-19 patients [32]. Differences in plasma angiotensin II levels in COVID-19 between our study and others could be due to SARS-CoV-2 viral load, severity of infection and extent of cell injury, ACE/ACE2 genotypes of patients [33, 34], or other mechanisms. In addition, elevated

plasma ACE2 in our patients might have mitigated any increase in circulating angiotensin II, via cleavage to angiotensin-(1–7).

ARBs and ACEi may alter plasma RAS component levels and expression in tissues (e.g. increased ACE2 expression in cardiac tissue in patients with heart disease [35] and in ARB-treated animals [36]). In our study, plasma ACE2 was significantly lower in patients who were on ACEi compared to those not on ACEi and in ACEi-exposed vs. ARB-exposed patients. Similarly, in patients with heart failure, ARB and ACEi use is associated with lower plasma ACE2 levels [36]. Our data support the hypothesis that inhibition of local angiotensin II formation may reduce tissue injury/inflammation and prevent shedding of soluble ACE2 fragments into the circulation.

Male sex has been identified as a risk factor for mortality in COVID-19 [37, 38]. In adjusted analysis, we found that males with COVID-19 had significantly higher plasma levels of ACE than females. In healthy subjects, serum ACE activity did not differ between sexes, but was lower in older men compared to younger men [39]. Recent studies in cultured human airway smooth muscle cells demonstrate higher ACE2 expression levels in males than females, suggesting a possible link between sex differences and outcomes with COVID-19 [40]. While we did not find an association between plasma ACE (or ACE2) and mortality in COVID-19, the sample size was relatively small and analysis of larger cohorts is needed to address this possibility.

Similarly, adjusted analysis showed that plasma angiotensin I levels were lower in ventilated than non-ventilated acute COVID-19-infected patients in our study, a finding of unclear significance. In patients with ARDS, Reddy et al. reported higher plasma angiotensin I levels in non-survivors than survivors, using a liquid chromatography-mass spectrometry assay method [22]. Survivors of ARDS had higher median angiotensin-(1–9)/angiotensin I and angiotensin-(1–7)/angiotensin I ratios than non-survivors, suggesting higher ACE2 activity.

Strengths of our study include the repeated plasma sampling for RAS components in acute COVID-19 hospitalized patients over 14 days, the multicenter design enhancing generalizability, healthy controls for comparison, and assessment of multiple RAS components to characterize the response to early acute COVID-19. One limitation is that we measured only five RAS components in plasma, and did not include renin or other peptides such as angiotensin-(1–9). We measured plasma levels at baseline and over 14 days and cannot discern effects of COVID-19 on RAS at later times. Another limitation is the lack of information on possible comorbidities in the control group, although it is assumed that they were in good health, as blood donors. Finally, future studies should evaluate plasma levels of RAS components early in the course of COVID infection to determine when in the pre-hospital course changes occur.

In summary, the RAS is altered in COVID-19, with significant increases in plasma levels of ACE, ACE2 and angiotensin-(1–7). RAS dysregulation persists for at least 14 days after hospitalization for acute COVID-19. Clinical trials addressing the therapeutic benefits of ARB or ACEi use in COVID-19 are increasingly justified by our findings that ACEi-exposed patients had significantly lower plasma ACE2 and angiotensin II than ACEi-not exposed and

compared to ARB-exposed patients. Our data also suggest that trials of ARBs and ACEi should include study drug exposure for at least 14 days.

Methods

Research Ethics

This study was conducted in accordance with all institutional regulations and guidelines at the University of British Columbia, as well as the Declaration of Helsinki and clinical trials guidelines from the Canadian Institutes of Health Research, and was approved by the Providence Health Care and University of British Columbia Human Research Committee. The committee determined that the study was in a category of minimum risk, since it was an observational cohort of anonymized data collected clinically and used discarded plasma from routine clinical laboratory tests. Therefore informed consent was

deemed not necessary for this research. The study was also approved by the ethics committees of all participating institutions based on this minimal risk criteria, and there was deemed no need for informed consent.

Patient Selection Criteria

Individuals greater than 18 years of age with confirmed COVID-19 infection (according to local hospital or provincial laboratories clinically approved testing for SARS-CoV-2) who were admitted to hospital were included. There were no exclusion criteria. Control human plasma was obtained from whole blood donors (Human Plasma K₂EDTA, BioIVT, Hicksville, NY, USA).

Baseline Characteristics of Controls and COVID-19 Patients

We recorded age and sex in controls, and baseline characteristics (at study enrollment) of COVID-19 patients (age, sex, and presence of heart failure, hypertension, chronic kidney disease, and diabetes, the commonest co-morbidities of COVID-19 [5, 6, 7] associated with increased risk of ICU admission [7]) and 28-day mortality.

Measurement of Plasma RAS Pathway Components

EDTA-plasma was obtained from discarded clinical blood samples from patients at baseline and on days 2, 4, 7 and 14 after hospitalization. Control human plasma samples were obtained from blood donors, as described above. Specimens were frozen at -80 °C. A panel of RAS components in plasma was measured by enzyme-linked immunosorbent assays (ELISAs), with each assay performed in duplicate, using commercial suppliers: ACE (MyBioSource Inc., cat. # MBS9359476, San Diego, CA, USA); ACE2 (R&D Systems cat. # DY933-05, Oakville, ON, Canada), angiotensin I (MyBioSource Inc., cat. # MBS262759); angiotensin II (Enzo Life Sciences, Inc., cat. # ADI-900-204, Farmingdale, NY, USA), and angiotensin-(1–7) (Cusabio, cat. # CSB-E14242H, Wuhan, China). Standard curves were prepared for each 96-well plate, and intra-assay CVs ranged between 4.97% and 9.22% for all assays.

Statistical Analyses

Plasma levels of RAS components were not normally distributed, and non-parametric statistics were used for analysis. Spearman correlation (rho) was used to determine correlations between the plasma levels of RAS peptides and enzymes. We compared plasma RAS component levels of controls to the baseline levels in COVID-19 patients.

Wilcoxon rank sum test was used in unadjusted analyses of COVID-19 vs. controls, and within COVID-19 for comparisons of survivors vs. non-survivors at day 28, ventilated vs. not ventilated (invasive ventilation), males vs. females, and ARB and ACEi-exposed vs. not exposed. For COVID-19 patients, adjusted analyses based on quantile regression were performed to compare the median plasma RAS component levels between the aforementioned groups after adjustment for age, sex, chronic cardiac disease, chronic kidney disease, diabetes and hypertension. Adjusted comparisons were not performed for the ARB group due to low number of patients (n = 4). Further analysis was conducted to compare ACEi-exposed vs. not exposed with the ARB-exposed patients being classified as ACEi-not exposed. Median plasma RAS component levels over time were modelled using linear quantile mixed regression with patient-specific random intercept. Time was included as a categorical variable in the model and changes in median plasma RAS component levels relative to baseline were assessed by appropriate regression coefficients from the estimated model.

Declarations

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AUTHOR CONTRIBUTIONS

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Figures

Figure 1

Median and 25-75% box plots of RAS components in COVID-19 and healthy controls by survival, use of ventilation, sex and use of ARBs and ACE inhibitors. P values according to Wilcoxon rank sum test.

Figure 2

Estimated change in median RAS components from baseline and 95% confidence intervals based on linear quantile mixed regression in COVID-19.

Figure 3

SARS-CoV-2 causes cell injury, necrosis and tissue inflammation leading to release into the circulation of ACE and ACE2, and subsequent conversion of angiotensin II to angiotensin-(1-7). Thus, plasma ACE, ACE2 and angiotensin-(1-7) are increased in COVID-19.

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