Hospitalized Patients With COVID-19 Have Higher Plasma Aldosterone-Renin Ratio and Lower ACE Activity Than Controls

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Abstract

Context: SARS-CoV-2 infects cells via the angiotensin converting enzyme 2 (ACE2) receptor, whose downstream effects “counterbalance” the classical renin angiotensin aldosterone system (RAAS).

Objective: We aimed to determine to what extent circulating RAAS biomarker levels differ in persons with and without COVID-19 throughout the disease course.

Methods: We measured classical (renin, aldosterone, aldosterone/renin ratio [ARR], Ang2, ACE activity) and nonclassical (ACE2, Ang1,7) RAAS biomarkers in hospitalized COVID-19 patients vs SARS-CoV-2 negative controls. We compared biomarker levels in cases with contemporaneous samples from control patients with upper respiratory symptoms and a negative SARS-CoV-2 PCR test. To assess RAAS biomarker changes during the course of COVID-19 hospitalization, we studied cases at 2 different times points ~ 12 days apart. We employed age- and sex-adjusted generalized linear models and paired/unpaired t tests.

Results: Mean age was 51 years for both cases (31% women) and controls (50% women). ARR was higher in the first sample among hospitalized COVID-19 patients vs controls (P=0.02). ACE activity was lower among cases at their first sample vs controls (P= <0.001). ACE2 activity, Ang 1,7, and Ang2 did not differ at the 2 COVID-19 case time points and they did not differ in COVID-19 cases vs controls. Additional adjustment for body mass index (BMI) did not change our findings.

Conclusions: High ARR, independent of BMI, may be a risk marker for COVID-19 hospitalization. Serum ACE activity was lower in patients with COVID-19 vs controls at the beginning of their hospitalization and then increased to similar levels as controls, possibly due to lung injury, which improved with inpatient disease management.

Key Words: COVID-19, renin-aldosterone ratio, hospitalization, severity, renin-angiotensin aldosterone system, obesity, ACE-2, biomarkers

Abbreviations: ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Ang, angiotensin; Ang1,7, angiotensin 1,7; Ang2, angiotensin 2; ARB, angiotensin 2 receptor blocker; ARR, aldosterone/renin ratio; BMI, body mass index; RAAS, renin angiotensin aldosterone system; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ZSFGH, Zuckerberg San Francisco General Hospital.

Several observations about the pathophysiology and epidemiology of the SARS-CoV-2 pandemic suggest a key role of the renin angiotensin aldosterone system (RAAS) in COVID-19 disease [1]. On the classical arm of the RAAS pathway, renin mediates conversion of angiotensinogen to angiotensin (Ang) (Fig. 1). Angiotensin converting enzyme (ACE) mediates conversion of Ang to angiotensin 2 (Ang2). ACE is primarily localized in the lungs and to a lesser degree in the kidney. Ang2 has a number of cardiac and vascular effects including acting on the adrenal cortex to release aldosterone. Aldosterone acts on the kidneys to stimulate sodium and fluid retention, thereby elevating blood pressure. On the counterbalance RAAS pathway, angiotensin converting enzyme 2 (ACE2) converts Ang1 and Ang2 to Ang 1,9 and Ang 1,7 respectively (Fig. 1). The latter...
acts via the Mas receptor to downregulate aldosterone and exert other protective effects on the heart and blood vessels [1].

SARS-CoV-2 virus leverages the membrane-bound RAAS counterregulatory protein, ACE2 cellular receptor, to infect its host [2–4]. ACE2 is a homolog of ACE and counteracts the adverse cardiovascular effects of RAAS activation [5]. Membrane-bound ACE2 negatively regulates RAAS by converting Ang2 to vasodilatory Ang1-7, diminishing and opposing the vasoconstrictor effect of Ang2 [2]. There is differential tissue expression of ACE2 in heart, kidneys, and lungs of healthy patients, and its expression in the kidneys and lungs is positively correlated with age [6]. Circulating ACE2 levels were higher among patients with obesity and diabetes in 2 large cohorts of patients with atrial fibrillation [7]. In addition to older age and male sex, risk factors for COVID-19 hospitalization include obesity, diabetes, cardiovascular disease, and possibly hypertension [8]. A recent investigation demonstrated that higher ACE2 levels on hospital admission was associated with worse outcomes in COVID-19 [9]. Other RAAS markers were not studied [9].

Thus, the effects of SARS-CoV2 on circulating RAAS biomarkers in COVID-19 are not fully understood. Therefore, we undertook a study to compare levels of circulating RAAS biomarkers (renin, Ang2, ACE, aldosterone, aldosterone/rein ratio [ARR], ACE2, Ang 1,7) among persons not on RAAS blockers in: (1) Patients hospitalized with COVID-19 and (2) patients who received a respiratory viral panel for upper respiratory symptoms but who were negative for SARS-CoV-2 on PCR testing. We hypothesized that circulating RAAS markers may differ in patients with COVID-19 vs non-COVID-19-related respiratory infections and that there may be measurable differences in these RAAS markers at the start and end of a hospitalization for COVID-19.

Materials and Methods
Study Sample, Clinical Data, and Specimens
The study was designed as a case-control study. The primary analysis utilized remnant plasma samples from routine clinical laboratory testing at Zuckerberg San Francisco General Hospital (ZSF GH). All participants who were cases tested positive for SARS-CoV-2 by real-time polymerase chain reaction (RT-PCR) in nasopharyngeal swabs. Controls were selected from a biospecimen repository of patients who had been tested with a respiratory viral sample for upper respiratory symptoms and who were confirmed to be COVID-19 negative (seen in emergency department, inpatient, or outpatient settings). Clinical data was extracted from electronic health records and included demographic information, major comorbidities, patient-reported symptom onset date, symptoms, and indicators of disease severity. We selected men and women who were > age 18 years, hospitalized for COVID-19 at ZSF GH with stored samples, and had a length of stay between 6 and 20 days. Since we were interested in comparisons of RAAS biomarker levels at the beginning and end of the COVID-19 infection, we limited reported symptom onset to ≤7 days before sample collection and included samples with adequate volume. In order to reflect meaningful differences in RAAS case biomarkers between the first and second sample, we selected a minimum time difference of 6 days between the 2 serial samples. We also excluded longer lengths of stay to exclude the effects of prolonged hospital stays on the biomarkers of interest, setting our maximum length of stay to 20 days. We excluded persons with medical record–based diagnoses of chronic kidney disease, coronary artery disease, congestive heart failure, HIV, liver disease, solid organ transplant, and those on medications that directly block RAAS system (including ACE inhibitors, angiotensin 2 receptor blockers, and/or aldosterone antagonists). A serum sample was collected during routine phlebotomy, and remnant samples (after the ordered clinical tests were completed) were stored in the ZSF GH Lynch laboratory biospecimen repository. This study was approved by the Institutional Review Board of the University of California, San Francisco. The committee judged that written consent was not required for use of remnant specimens.

Clinical Data Collection
Demographic and clinical data were abstracted from the electronic medical record via clinical documents, problem lists,
and medication lists. Blood pressure measurements were done at the time of hospital or emergency department admission for both cases and controls.

Serum was stored from discarded clinical samples used for routine laboratory measurements. ACE2 (Human ACE-2 DuoSet ELISA, #DY93305, R&D Systems, Minneapolis, MN, RRID: AB_355722), Ang2 (Human Angiotensin II (Competitive EIA) ELISA Kit, #LS-F23421, LSBio, Seattle, WA, RRID: AB_2921262), Ang1,7 (Human Angiotensin 1,7 ELISA Kit, #NPB2-69078, Novus Biologicals, Littleton, CO, RRID: AB_2921261), aldosterone (Aldosterone Parameter Assay Kit, #KGE016, R&D Systems, Minneapolis, MN, RRID: AB_2750856), and renin (Human Renin Quantikine ELISA Kit, #DRENO0, R&D Systems, Minneapolis, MN, RRID: AB_2750857) levels were measured using commercially available enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer’s instructions. ACE activity was measured by a kinetic enzymatic assay (ADVIA1800; Siemens, Munich, Germany). All biomarker data was log transformed because of skewed data.

Statistical Methods
We compared both classical (renin, Ang2, ACE1, aldosterone, ARR) and counterregulatory (ACE2, Ang 1,7) RAAS biomarkers among hospitalized COVID-19 patients at 2 time points during hospitalization and among SARS-CoV-2 negative controls. Among COVID-19 hospitalized cases, we compared RAAS biomarkers on samples at 2 different time points: (1) close to admission and (2) close to hospital discharge. We additionally compared RAAS biomarker levels in cases with contemporaneous samples from control patients who had received a respiratory viral panel for upper respiratory symptoms and who were negative for SARS-CoV-2. In order to detect differences between the 3 groups in a single model, mixed linear models were utilized (adjusted for age and sex and centered on sex). First and second case samples were compared using paired t tests. Cases and controls were compared using unpaired t tests. All biomarker data were log transformed because of skewed data. Data were adjusted for age and sex (and also centered on sex) in order to exclude confounding by these factors. In secondary analysis, ACE2/ACE ratio was assessed. We also additionally adjusted all models for body mass index (BMI; there was missing BMI data for 1 patient in the case group and 6 patients in the control group). Given that this is hypothesis-generating research, we did not adjust our P value thresholds for multiple comparisons.

Results
Descriptive Characteristics
We examined plasma RAAS biomarkers in n = 32 cases and n = 32 controls. Table 1 shows the descriptive characteristics of the study population. Briefly, mean age was 51 years of age in both cases and controls. There were fewer women cases (31%) compared to controls (50%). Among cases, 72% were Hispanic, 15.6% Asian, 9.4% Black, and 3% White. Among controls, 47% were Hispanic, 6.3% Asian, and 34.4% White. Diabetes was present in 31% of cases vs 19% of controls. Hypertension prevalence was the same in cases and controls (25%), and mean blood pressure was similar in cases and controls (∼138/80 mmHg). Controls were on average in the overweight category (BMI > 25 and <30 kg/m²) and cases, in the obese category (BMI > 30 kg/m²). Patients with COVID-19 were more likely have been admitted to the intensive care unit and to have been on mechanical ventilation compared with patients in the control group. The average nontransformed RAAS biomarker levels in cases and controls are noted, with their respective median and interquartile ranges, in Table 1.

Classical RAAS Biomarkers in COVID-19 Cases vs Controls
In mixed models, ARR was significantly higher among hospitalized COVID-19 patients vs controls. The ARR was higher in the first COVID-19 sample vs controls (P = 0.03) and in the second COVID-19 sample vs controls (P = 0.02) (Fig. 2A). Aldosterone levels were higher in cases vs controls, and this was true at both the first (P = 0.003) and second (P = 0.004) time points (Fig. 2B). Renin levels were higher in cases in the initial sample vs controls, although this was of borderline statistical significance (P = 0.06) (Fig. 2C). Renin levels were similar at the 2 time points in cases (Fig. 2C). ACE activity was lower among cases at their first sample vs controls (P = <0.001) (Fig. 3A).

Counterregulatory RAAS Biomarkers in COVID-19 vs Controls
Ang 1,7, Ang2, and ACE2 were neither different at the 2 COVID-19 case time points, nor did they differ in COVID-19 cases vs controls (all P values nonsignificant) (Fig. 4A and 4B, Fig. 3B). We assessed the ratio of Ang1,7/Ang2 (an alternative measure of ACE2 activity), and this did not differ in cases vs controls and did not differ among samples at the 2 time points in cases (Fig. 4C).

Secondary Analysis
We assessed ACE2 activity/ACE activity ratio, and although this was higher in cases vs controls, this finding did not reach statistical significance (Fig. 3C). Additional adjustment for BMI did not materially affect the results for the difference in ARR, including the statistical significance of the result (P = 0.03); (Supplementary Fig. 1A) [10]. Upon additional adjustment for BMI, ACE activity remained significantly lower among cases at their first sample vs controls (P = 0.004); (Supplementary Fig. 2) [10]. Upon additional for BMI, none of the counterregulatory RAAS biomarkers nor ratio of Ang1,7/Ang2 differed at the 2 COVID-19 case time points, nor did they differ among cases or controls (data not shown).

Discussion
Primary Findings
In this study of 64 men and women (n = 32 persons hospitalized with COVID-19 and n = 32 controls) we found that (1) ARR was significantly higher among hospitalized COVID-19 patients vs controls; (2) aldosterone levels were higher in cases vs controls; (3) ACE activity was lower among COVID-19 cases at the beginning but not at the end of hospitalization as compared with controls; (4) ACE2 activity, Ang 1,7, and Ang2 were similar in the 3 comparison samples; (5) ACE2/ACE ratio was higher in cases vs controls (although these differences did not reach statistical significance); and (6) additional adjustment for BMI in a subset of patients with available data did not materially change our results.
Table 1. Sample characteristics and RAAS biomarker levels in cases of patients hospitalized with COVID-19 and controls with respiratory symptoms but no COVID-19

|                      | CASES N = 32 | CONTROLS N = 32[^a] |
|----------------------|-------------|---------------------|
| **Age**              | 51 ± 6      | 51 ± 18             |
| **Women**            | 10 (31%)    | 16 (50%)            |
| Menopause            | 6 (60% of women) | 6 (38% of women)   |
| Hormone therapy      | 0           | 0                   |
| **Race/Ethnicity**   |             |                     |
| Asian                | 5 (15.6%)   | 2 (6.3%)            |
| Black                | 3 (9.4%)    | 3 (9.4%)            |
| Hispanic             | 23 (72%)    | 15 (47%)            |
| White                | 1 (3%)      | 11 (34.4%)          |
| Other                | 0           | 1 (3.1%)            |
| **Hypertension**     | 8 (25%)     | 8 (25%)             |
| Systolic BP[^b]      | 137.6 ± 21  | 138 ± 27            |
| Diastolic BP[^b]     | 80 ± 14.2   | 83 ± 16.4           |
| Body mass index, kg/m^2 | 31.5 ± 5.5  | 26.3 ± 5.8          |
| **Potassium**        | 4.0 ± 0.47  | 3.99 ± 0.45         |
| **Diabetes**         | 10 (31%)    | 6 (19%)             |
| Outpatient[^c]       | 0           | 15 (44%)            |
| **Length of stay**   | 14 ± 6.3    | 2.9 ± 3.8           |
| Health insurance, yes| 27 (84%)    | 26 (93%)            |
| Mechanical ventilation| 17 (53%)   | 1 (3%)              |
| Admitted to intensive care unit | 8 (25%) | 1 (3%) |
| Transferred to intensive care unit | 3 (9.4%) | 0 |
| **Diuretic**         | 0           | 2 (6.5%)            |
| Calcium channel blocker | 3 (9.4%) | 1 (3.1%) |
| Beta blocker         | 3 (9.4%)    | 3 (9.4%)            |
| Aspirin              | 0           | 3 (9.4%)            |

*Non-log-transformed RAAS mediator levels (median, interquartile range)*

|                      | CASES | CONTROLS |
|----------------------|-------|---------|
| Time between 2 samples, days | 12.0 ± 10.0 | N/A     |
| Aldosterone to renin ratio, ug/(L/h) per pmol/L | | |
| Sample 1              | 0.04 (0.01–0.10) | 0 (0–0.36) |
| Sample 2              | 0.05 (0.007–0.21) | |
| Renin, pg/mL          |       |         |
| Sample 1              | 1600 (936–3209) | 958 (474–2107) |
| Sample 2              | 1221 (752–2751) |     |
| Aldosterone, pg/mL    |       |         |
| Sample 1              | 98.08 (17.15–316.85) | 0 (0–345.20) |
| Sample 2              | 113.54 (12.50–277.78) |     |
| Ang2, pg/mL           |       |         |
| Sample 1              | 0.51 (0.34–1.73) | 0.61 (0.24–1.08) |
| Sample 2              | 1.05 (0.24–2.51) |     |
| ACE, mU               |       |         |
| Sample 1              | 27.7 (15.2–38.9) | 44.9 (29.8–58.2) |
| Sample 2              | 36.2 (26.65–56.55) |     |
| ACE2, pg/mL           |       |         |
| Sample 1              | 2.61 (0.67–4.80) | 0.80 (0.34–17.91) |
| Sample 2              | 1.81 (0.71–6.79) |     |
| Ang1,7, pg/mL         |       |         |
| Sample 1              | 74.22 (39.50–204.08) | 96.60 (41.22–149.28) |
| Sample 2              | 108.78 (39.68–191.87) |     |

(continued)
In Context of Prior Studies
Studies of circulating RAAS markers in COVID-19 disease are limited. In a recent study of patients with COVID-19 vs propensity matched controls, Ang and Ang2, as well as Ang 1,7 were lower in persons with COVID-19 vs controls [11]. In this study, ACE and ACE2 were similar in the 2 groups of patients [11]. However, in this study, no indication was made of the timing of samples relative to admission/discharge and/or death [11]. Aldosterone was not measured in the prior study.

ARR in Primary Hyperaldosteronism
Some conditions marked by high aldosterone can lead to suppression of renin via a negative physiologic feedback mechanism. Thus, a high aldosterone to renin ratio (or ARR) is the primary diagnostic tool for primary hyperaldosteronism (caused by adrenal hyperplasia, adenoma, or is idiopathic). Primary hyperaldosteronism (ARR generally at least greater than 30 ng/dL per μg/L/h) [12] accounts for 8% of hypertension and from 6% to 39% of treatment-resistant hypertension; it is more prevalent than previously appreciated [13, 14]. High aldosterone levels in the normal range precede the development of essential hypertension [4].

ARR in Secondary Hyperaldosteronism
Elevation of ARR can also result from secondary aldosteronism and is often detected in individuals with increased cardiometabolic risk factors, including hypertension, insulin resistance, and obesity. Adipose tissue is a renin-independent depot for aldosterone, thus providing causal underpinnings between obesity and states of secondary hyperaldosteronism. Recent data also suggest that there may be a continuum of renin-independent aldosterone production that parallels the severity of hypertension [15]. Indeed, a recent study of 1000 persons across 4 academic medical centers proposes a redefinition of primary aldosteronism syndrome, implicating it in the pathogenesis of “essential” hypertension [15].

Cardiometabolic Risk Factors, Salt-Sensitive Hypertension, and COVID-19 Severity
Whereas diabetes and congestive heart failure (for which hypertension is a risk factor) are both protective for developing non-COVID-19 acute respiratory distress syndrome [16, 17], both diabetes and hypertension are risk factors for intensive care unit stay, mechanical ventilation, and death in COVID-19 [18–20]. Studies have now demonstrated that obesity is a prevalent factor among hospitalized patients, and obesity is also related to mortality in COVID-19. Risk stratification for COVID-19 severity within the broader realm of hypertension and obesity may be important [8]. Thus, our findings of a higher

Table 1. Continued

|                        | CASES N = 32                  | CONTROLS N = 32\(^a\)            |
|------------------------|-------------------------------|---------------------------------|
| Sample 1               | 159.68 (38.99–369.16)         | 178.23 (105.166–462.3)          |
| Sample 2               | 112.046 (44.72–605.86)        |                                 |

\(^a\) n = 9 with no virus detected, n = 7 rhinovirus, n = 3 RSV, n = 2 human metapneumovirus, n = 2 coronavirus HKV1, n = 1 parainfluenza virus

\(^b\) Measurements were on admission.

\(^c\) n = 9 seen in emergency department

Figure 2. (A) Box and whisker plots of log aldosterone to renin ratio. (B) Box and whisker plots of log aldosterone. (C) Box and whisker plots of log renin.
ARR among persons with hospitalized COVID-19 (who were on average in the obese adipose category by BMI) also raise the possibility that those with obesity-related, salt-sensitive hypertension may be particularly at high risk for severe COVID-19. Indeed, salt-sensitive hypertension, often found in obese individuals, is a key mechanism in obesity-related secondary hyperaldosteronism. The extent to which persons with higher ARR have a dysregulated response to SARS-CoV-2 infection should be further examined. Indeed, further studies examining the interplay between RAAS biomarkers, including ARR, and COVID-19 progression may provide important insights into the pathophysiology of how cardiometabolic risk factors predispose to severe forms of COVID-19.

ACE and ACE2 in COVID-19

In COVID-19, ACE2 gene expression in areas of infected lung tissue has been demonstrated to increase dramatically when
compared to noninfected lung tissue [21], and separate studies have demonstrated vasodilation in areas of COVID-19 pneumonia vs uninfected lung tissue [22]. Taken together, these 2 observations raise the possibility that there is a unique pathophysiology of COVID-19 acute respiratory distress syndrome in which local vasodilatory effects of ACE2 occur in the body via soluble ACE2 receptor release and/or membrane-bound ACE2 activation. Meanwhile, there may be vasoconstriction in noninfected tissue causing less blood flow to unaffected areas and more blood flow to infected tissue, resulting in ventilation/perfusion mismatch. We did not demonstrate that circulating plasma ACE2 activity differed in COVID-19 patients when compared to control patients. One prior study demonstrated higher ACE2 levels predicted worse outcomes in patients with COVID-19 [9]. We recognize that study of lung tissue RAAS biomarkers in SARS-CoV-2 would have likely been more relevant than circulating ACE2. We are uncertain why ACE levels were lower in COVID-19 and were shown to rise with treatment in the hospital, as compared with controls. It is possible that severe acute lung injury such as that caused by SARS-CoV-2 infection lowers ACE levels. If COVID-19 causes measurable destruction of pulmonary capillary endothelial cells, this could account for decreased ACE production at the beginning of COVID-19 in cases which may improve with medical treatment and stabilization. The improvement could theoretically result in higher ACE activity at the end of hospitalization, which we demonstrated was equivalent to ACE activity in the control group.

Limitations
Cases were limited to those hospitalized with COVID-19 who survived to discharge within 20 days so there may be selection bias. We also excluded those expected to have abnormal RAAS markers based on past medical history and use of RAAS blockade, which limits the generalizability of our findings. We did not have pre- or postconvalescent samples upon which to compare RAAS biomarker levels, which limits conclusions. We were not able to measure tissue levels of RAAS mediators. We used discarded clinical specimens, which may be suboptimal study samples in terms of their handling. Our controls were from a contemporaneous convenience sample. Sampling conditions were not perfectly controlled and this may have added some degree of misclassification to the biomarker levels (if this misclassification was random, this may have biased our estimates toward the null value). Given that this was a hospitalized cohort at the beginning of the pandemic (when clinical research involving direct patient contact was restricted), tighter control of sampling conditions was not possible. We collected blood pressure and medications on admission and did not account for potential changes during hospitalization (except for ACE inhibitors and angiotensin 2 receptor blockers, which no study patient was on at any time during the study period).

Directions for Future Research
Studying tissue levels of RAAS mediators during the onset of COVID-19 is an important next step. Study of preconvalescent plasma ARR as a risk biomarker for hospitalized COVID-19 is another important future direction and may help to uncover whether ARR can be used to risk stratify patients with cardiometabolic risk factors (including hypertension) in terms of their relative risk for severe forms of COVID-19, requiring hospitalization. Targeted study of serial RAAS mediators, such as ACE2 and ACE activity, at several time points across the course of illness may help our understanding of COVID-19 pathophysiology. Our findings may have implications on mechanisms by which persons with cardiometabolic risk factors are at high risk for severe COVID-19. Further studies of RAAS biomarkers in COVID-19 may help uncover these pathways.

Conclusion
In conclusion, in this case-control study of adults hospitalized with COVID-19 compared to those with non-SARS-CoV-2 acute respiratory illness we found important differences in circulating RAAS biomarkers, including elevated ARR and aldosterone, as well as changes over time from the beginning to end of hospitalization among cases. These findings may provide mechanistic explanations for the associations of hypertension and obesity with adverse COVID-19 outcomes.

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Disclosures
P.G. serves on the Medical Advisory Board of SomaLogic Inc., for which he accepts no salary or renumeration of any kind.

Data Availability
Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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