Current Topics in Virology
Vol. 18, 2021

Original Article

Clinical spectrum of COVID-19 and plasma angiotensin II levels

Ume L. Abbas[1,2,*], An-Lin Cheng[2], Shital M. Patel[1], Sidra Younus[3], Gustavo A. Rivero[1], Todd M. Lasco[1] and Mayar Al Mohajer[1]

[1]Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030;
[2]University of Missouri–Kansas City, 2411 Holmes St, Kansas City, MO 64108;
[3]Houston Methodist Hospital, 6565 Fannin St, Houston, TX 77030, USA.

ABSTRACT

Angiotensin II levels in COVID-19 are controversial. We studied 12 hospitalized patients, including their baseline levels of peripheral lymphocyte subsets (*via* flow cytometry) and plasma angiotensin II (*via* radioimmunoassay). Controls comprised radioimmunoassay's 124 healthy subjects. Angiotensin II levels (pg/ml) were elevated among patients versus controls (Mean ± standard deviation: 98.8 ± 146.9 versus 23.7 ± 15.6, $p < 0.0001$; Median, interquartile range: 27, 20 to 116 versus 22, 14 to 28). Half the patients had lymphocytopenia (< 1000 cells/mm^3), and the CD3+/CD4+ counts were negatively associated with body mass index, viral load, hospital stay and non-home discharge. Angiotensin II imbalance appears to be a biomarker for COVID-19 morbidity and merits further investigation.

KEYWORDS: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), renin-angiotensin-aldosterone system (RAAS), angiotensin-converting enzyme 2 (AngII), lymphocytopenia, biomarker.

INTRODUCTION

The role of renin-angiotensin-aldosterone system (RAAS) in the pathophysiology of coronavirus disease 2019 (COVID-19), is unclear [1]. The functional receptor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is angiotensin-converting enzyme 2 (ACE2), a primary enzyme within the counter-regulatory axis of the RAAS. In the classical system, renin cleaves angiotensinogen to form angiotensin I, which is converted to angiotensin II (AngII) by the angiotensin-converting enzyme (ACE). Conversely, ACE2 converts AngII, a vasoconstrictive and proinflammatory peptide, into counter-active angiotensin-(1-7). Increased activity of ACE-AngII relative to ACE2-Ang-(1-7) might drive the pulmonary and cardiovascular injury in COVID-19 [1]. However, the studies thus far have shown mixed results [2-7]. We sought to investigate further, AngII levels and their relevance in COVID-19.

MATERIALS AND METHODS

We conducted a retrospective review of patients hospitalized with COVID-19, referred to our care, from June 1 to June 14, 2020. Inclusion criteria included a positive reverse transcription polymerase chain reaction (RT-PCR) test for SARS-CoV-2 (*via* nasopharyngeal swab) on presentation, and admission to an intensive care or step-down unit. COVID-19 was classified, as mild, moderate, severe or critical, per the World Health Organization criteria [8]. Data were abstracted from the electronic medical records and included (Table 1)

*Corresponding author: abbasu@umkc.edu

Table 1. Patient characteristics, inpatient data and outcomes.

No	Age (years)	Gender	Race/ Ethnicity	BMI (kg/m^2)	Comorbidities	AHM	Severity	C_T value	TLC ($cells/mm^3$)	CD3 ($cells/mm^3$)	CD4 ($cells/mm^3$)	CD8 ($cells/mm^3$)	NK ($cells/mm^3$)	CD19 ($cells/mm^3$)	Ang II (pg/ml)	Therapy	LOS (days)	Outcome
1	47	female	white (NH)	28.4	DM, HTN	losartan	severe	27.8	1529	1127	880	250	147	239	34	remdesivir, steroids	6	home
2	74	female	black (NH)	25.5	DM, HTN	carvedilol	severe	29	1198	760	537	222	119	292	149	remdesivir	8	home
3	95	female	black (NH)	36.6	DM, HLD, HTN, obesity	spironolactone / HCTZ	moderate	NA	697	360	221	127	214	116	< 12	none	10	home-hospice
4	81	male	white (NH)	18.6	CAD, ESRD, HLD, HTN	carvedilol, losartan	severe	21.6	269	163	130	34	90	9	21	tocilizumab	8	death
5	88	female	black (NH)	36.3	CHF, CKD, DM, GERD, HLD, HTN, obesity	amlodipine, carvedilol, furosemide, hydralazine	severe	19.8	588	530	194	337	25	26	19	convalescent plasma, steroids	14	skilled nursing facility
6	47	female	white (NH)	39.3	HLD, HTN, hypothyroidism, obesity, PMR	Bisoprolol / HCTZ	critical	NA	932	540	288	257	57	315	>500	remdesivir, steroids	53	long-term acute care
7	59	male	white (H)	32.0	CAD, DM, ESRD, HTN, obesity	amlodipine, metoprolol XL, nifedipine	severe	24.3	879	674	565	108	88	108	198	tocilizumab	4	home
8	83	male	black	26.1	blindness, BPH, CAD, DM, HLD, HTN	lisinopril, terazosin	severe	31.7	810	476	361	120	123	206	83	none	3	home
9	86	female	black	30.3	GERD, HTN, IBS, migraine, obesity	clonidine, losartan, spironolactone	moderate	26.1	1989	1451	833	657	376	153	27	none	3	skilled nursing facility
10	35	female	white (H)	32.8	DM, HLD, obesity	NA	severe	27.6	1079	848	521	327	110	113	17	none	2	home
11	69	male	black	36.3	CAD, CHF, COPD, DM, ESRD, gout, HTN, OSA, obesity	carvedilol, ezetimibe / simvastatin, losartan, hydralazine	severe	39.9	2,524	1,751	884	865	661	99	NA	tocilizumab	15	skilled nursing facility
12	31	female	black	50.0	asthma, obesity	none	moderate	NA	2485	1821	1070	724	178	457	26	none	3	home

comorbid conditions, outpatient medications, laboratory data and length of stay (LOS). The laboratory data, based on the prevailing protocols and/or clinicians' discretion, encompassed various biomarkers, obtained at baseline (~ admission) and during follow-up. We focused on the baseline levels of peripheral lymphocyte subsets (measured *via* flow cytometry) and plasma AngII (measured *via* radioimmunoassay [RIA]; Quest Diagnostics). Controls comprised 124 subjects (healthy, normotensive, 61 females and 63 males, aged 18-65 years) of Quest's RIA (limit of quantification [LOQ]: 12-500 pg/ml; normal values: ≤ 52 pg/ml). Outcomes included hospital-death and discharge to home, skilled nursing facility or long-term acute care, and excluded re-admission and post-acute sequelae of COVID-19.

Data were analyzed using SPSS (version 25), applying Mann-Whitney U, Chi-square and Spearman's correlation tests, as appropriate. Student's t-test and box-plots were employed to compare the mean and median AngII values among patients and controls, respectively.

Approval for this study was obtained from the Baylor College of Medicine's institutional review board.

RESULTS

Twelve patients were included in our analyses (Table 1). Two patients (both female, aged 20 and 41 years, discharged to home after a 3- and 2-day stay, respectively) were excluded due to lack of laboratory data. The median age was 72 years (interquartile range [IQR]: 47-84 years). Women comprised 67% and most patients had severe or critical disease (75%). COVID-19-specific therapies included steroids (25%), remdesivir (25%), tocilizumab (25%) and convalescent plasma (8.33%). The median LOS was 7 days (IQR: 3-11 days). Seven patients (58.3%) discharged home, while four had non-home discharge. One patient died (8.3%).

Six patients had lymphocytopenia (< 1000 cells/ml), with decreased CD3+ CD4+ and/or CD8+ T cell counts. Contrastingly, AngII levels were elevated (> 52 pg/ml) in four patients. The highest level (> 500 pg/ml) was in a patient requiring prolonged intensive care unit stay (53 days) with acute respiratory distress syndrome and severe hypertension. AngII was normal (21 pg/ml) in the patient who died, albeit with severe lymphocytopenia. The AngII levels among patients were higher compared to controls (Figure 1).

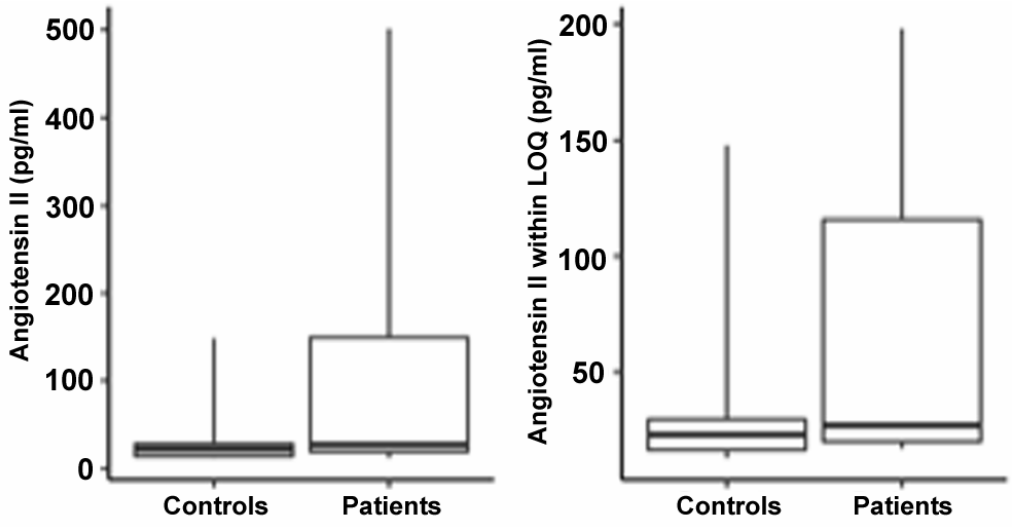

Figure 1. Plasma angiotensin II levels among healthy controls and COVID-19 patients.
Boxplots showing the median, interquartile range, minimum and maximum values of angiotensin II (pg/ml) among controls and patients. Left panel: using all data values (n = 124 versus n = 11). Right panel: using data values within the limit of quantification (n = 103 versus n = 9).

Mean [standard deviation (SD)] plasma AngII values for patients versus controls were 98.8 [146.9] versus 23.7 [15.6] pg/ml, $p < 0.0001$; and median [IQR] AngII values were 27 [20-116] versus 22 [14-28] pg/ml. Excluding the two patients and 21 controls with levels above or below LOQ, mean [SD] AngII values were 63.8 [66.5] versus 26.1 [16.1] pg/ml, $p < 0.0001$; and median [IQR] AngII values were 27 [21-83] versus 23 [16.5-29.5] pg/ml.

The patients' AngII levels were not correlated with other variables (shown in Table 1) nor associated with hospitalization outcomes. CD3+ cell counts were higher for LOS ≤ 7 versus > 7 days (median 988 versus 530 cells/mm^3; $p = 0.05$), while CD4+ percentages were higher for home versus non-home discharge (median 47% versus 34%; $p = 0.015$). RT-PCR cycle threshold values (~ inversely correlated with viral load) were positively correlated with CD4+ cell counts ($r = 0.609$; $p = 0.047$), while body mass indices (BMIs) were negatively correlated with CD4+ percentages ($r = -0.635$; $p = 0.026$).

DISCUSSION

We examined the characteristics, inpatient data, outcomes, select immune markers and AngII levels, in a cohort of hospitalized patients with moderate to critical COVID-19. The baseline AngII levels among patients were elevated, while the CD3+/CD4+ T cell counts were depressed and negatively associated with BMI, viral load, hospital stay and non-home discharge.

Similar to our study, lymphocytopenia, especially reduced CD4+ and CD8+ cell counts at hospital admission, have been found to predict COVID-19 disease progression [9]. Conversely, the studies to date have substantial qualitative and quantitative differences, in terms of the measured AngII levels among COVID-19 patients and controls [2-7].

Our findings are in agreement with two previous studies [2, 3]. Plasma AngII levels were elevated among 12 patients with COVID-19 (half having acute respiratory distress syndrome and lymphocytopenia) compared to eight healthy controls, and positively correlated (unconfirmed by us) with viral load and lung injury [2]. Of note, the AngII levels in this study (~ median 300 pg/ml among patients versus 100 pg/ml among controls), were considerably higher than ours (median 27 versus 22 pg/ml). Similarly, in a larger study of 82 non-hypertensive patients with mild, moderate and critical COVID-19 and 12 critically ill controls, the COVID-19 patients had elevated plasma AngII levels, that correlated with disease severity, and exceeded versus the controls [3]. The mean [SD] AngII levels (pg/ml) were 148 [25.5] for mild, and 190.3 [93.3] and 212.7 [85.7] for moderate and severe COVID-19, versus 135.1 [32.8] for critical controls. In contrast, our findings are in disagreement with some other studies that found similar [4, 5] or lower [6, 7] AngII levels, among patients compared to controls. Different methodologies and COVID-19-phenotypes [10] may explain this variance, including the use of enzyme-linked immunosorbent assay in the previous studies (except for liquid chromatography-mass spectrometry in [7]), which has low specificity and yields variable AngII concentrations, as opposed to RIA, employed by us [11]. In a prospective study of 22 patients with moderate or severe COVID-19 and 11 matched moderately ill controls, the median [IQR] AngII was 15.3 [8.0-31.1] pM (~ pg/ml), for subjects overall, using RIA [12]. Interestingly, there was a trend towards higher mean AngII levels (pM) ranging from moderately ill controls (~ 16.5), to moderately (~ 18.3) and severely (~ 24.4) ill COVID-19 patients, though these differences were not statistically significant in this pilot study [12].

CONCLUSION

This study found elevated AngII levels among hospitalized patients with COVID-19 compared to controls. Rigorous evaluation of the RAAS, using standardized methods and clinical trials, and including various components and pathways, could provide clarification on whether or not RAAS imbalance is a driver of the severity of COVID-19, and may yield clinically useful biomarkers and therapies [1].

Limitations of this study include its small sample size, retrospective design, external controls and limited RAAS evaluation. Whether high AngII levels in patients hospitalized with COVID-19 are from pre-existing health-conditions and/or SARS-CoV-2 is unclear. Nevertheless, AngII imbalance

seems to be a biomarker for morbidity in COVID-19. Further research into mutual effects of SARS-CoV-2 and RAAS is warranted.

SOURCE OF FUNDING

None.

CONFLICT OF INTEREST STATEMENT

MA receives honoraria from the University of Arizona Continuing Medical Education Office and Society for Healthcare Epidemiology of America, and is a committee/board member at latter, Infectious Disease Society of America and Certification Board of Infection Control and Epidemiology, Inc. SMP is a recipient of AIDS Education Training Center grant from the Health Resources and Services Administration. GAR receives consulting fees from the Karyopharm Therapeutics and Bristol Myers Squib. None for the remaining authors.

ABBREVIATIONS

AHM : antihypertensive medication
AngII : angiotensin II (normal range ≤ 52 pg/ml)
BMI : body mass index
BPH : benign prostatic hyperplasia
CAD : coronary artery disease
CD3 : CD3+ T lymphocytes, absolute (normal range 603-2990 cells/mm^3)
CD4 : CD3+CD4+ T lymphocytes, absolute (normal range 441-2156 cells/mm^3)
CD8 : CD3+CD8+ T lymphocytes, absolute (normal range 125-1312 cells/mm^3)
CD19 : CD19+ B lymphocytes, absolute (normal range 107-698 cells/mm^3)
CHF : congestive heart failure
CKD : chronic kidney disease
COPD : chronic obstructive pulmonary disease
C_T value : cycle threshold value; the number of reverse transcription polymerase chain reaction cycles needed to amplify viral RNA to reach a detectable level. Xpert Xpress SARS-CoV-2/Flu/RSV test (Cepheid, Sunnyvale, CA, USA), having limit of detection 131 copies/ml, was used in this study.
DM : diabetes mellitus
ESRD : end stage renal disease
GERD : gastroesophageal reflux disease
H : Hispanic
HCTZ : hydrochlorothiazide
HLD : Hyperlipidemia
HTN : hypertension
IBS : inflammatory bowel syndrome
LOS : length of stay
NA : not available
NK : CD16+ CD56+ natural killer cells, absolute (normal range 95-640 cells/mm^3)
NH : Non-Hispanic
OSA : obstructive sleep apnea
PMR : polymyalgia rheumatica
TLC : total lymphocyte count, absolute (normal range 1.32-3.57 x 10^3 cells/mm^3)
XL : extended release

REFERENCES

1. Sparks, M. A., South, A. M., Badley, A. D., Baker-Smith, C. M., Batlle, D., Bozkurt, B., Cattaneo, R., Crowley, S. D., Dell'Italia, L. J., Ford, A. L., Griendling, K., Gurley, S. B., Kasner, S. E., Murray, J. A., Nath, K. A., Pfeffer, M. A., Rangaswami, J., Taylor, W. R. and Garovic, V. D. 2020, Hypertension, 76, 1350-1367.
2. Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., Wang, Z., Li, J., Li., J, Feng, C., Zhang, Z., Wang, L., Peng, L., Chen, L., Qin, Y., Zhao, D., Tan, S., Yin, L., Xu, J., Zhou, C., Jiang, C. and Liu, L. 2020, Sci. China Life Sci., 63, 364-374.
3. Wu, Z., Hu, R., Zhang, C., Ren, W., Yu, A. and Zhou, X. 2020, Crit. Care, 24, 290.
4. Henry, B. M., Benoit, S., Lippi, G. and Benoit, J. 2020, Prog. Cardiovasc. Dis., 63, 702-703.
5. Rieder, M., Wirth, L., Pollmeier, L., Jeserich, M., Goller, I., Baldus, N., Schmid, B., Busch, H. J., Hofmann, M., Kern, W., Bode, C., Duerschmied, D. and Lother, A. 2021, Am. J. Hypertens., 34(3), 278-281.
6. Ozkan, S., Cakmak, F., Konukoglu, D., Biberoglu, S., Ipekci, A., Akdeniz, Y. S., Bolayirli, I. M., Balkan, I. I., Dumanli, G. Y.

and Ikizceli, I. 2021, Crit. Care Med., 49(6), e613-e623.
7. Valle Martins, A. L., da Silva, F. A., Bolais-Ramos, L., de Oliveira, G. C., Ribeiro, R. C., Pereira, D. A. A., Annoni, F., Diniz, M. M. L., Silva, T. G. F., Zivianni, B., Cardoso, A. C., Martins, J. C., Motta-Santos, D., Campagnole-Santos, M. J., Taccone, F. S., Verano-Braga, T. and Santos, R. A. S. 2021, ERJ Open Res., 7(3), 00114-2021.
8. COVID-19 Clinical Managment: Living guidance 25 January 2021. Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1
9. Zhang, X., Tan, Y., Ling, Y., Lu, G., Liu, F., Yi, Z., Jia, X., Wu, M., Shi, B., Xu, S., Chen, J., Wang, W., Chen, B., Jiang, L., Yu, S., Lu, J., Wang, J., Xu, M., Yuan, Z., Zhang, Q., Zhang, X., Zhao, G., Wang, S., Chen, S. and Lu, H. 2020, Nature, 583(7816), 437-440.
10. DeMerle, K., Angus, D. C. and Seymour, C. W. 2021, JAMA, 325, 2041-2042.
11. Chappell, M. C., Pirro, N. T., South, A. M. and Gwathmey, T. M. 2021, Hypertension, 77, e29-e31.
12. Files, D. C., Gibbs, K. W., Schaich, C. L., Collins, S. P., Gwathmey, T. M., Casey, J. D., Self, W. H. and Chappell, M. C. 2021, Am. J. Physiol. Lung Cell Mol. Physiol., 321, L213-l218.