Circulating level of Angiopoietin-2 is associated with acute kidney injury in coronavirus disease 2019 (COVID-19)

Brandon Michael Henry1 · Maria Helena Santos de Oliveira2 · Isaac Cheruiyot3 · Justin L. Benoit4 · David S. Cooper1,5 · Giuseppe Lippi6 · Timothy D. Le Cras5,7 · Stefanie W. Benoit5,8

Received: 9 January 2021 / Accepted: 8 March 2021 / Published online: 23 March 2021 © The Author(s), under exclusive licence to Springer Nature B.V. 2021

To the editor,

Emerging evidence suggests that endothelial dysfunction plays a central role in the pathophysiology of coronavirus disease 2019 (COVID-19). Recent post-mortem studies have documented extensive endothelial damage and inflammatory infiltrates in pulmonary and extra-pulmonary capillary beds of COVID-19 patients [1, 2]. This results in loss of endothelial integrity, activation of pro-coagulant pathways, disruption of the alveolar-capillary barrier, and vascular hyperpermeability [2]. Endothelial damage is a common denominator of thrombosis (micro- and macrovascular), acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), and multiorgan failure (MOF), which are major drivers of morbidity and mortality in COVID-19 patients [3]. AKI is a common feature of COVID-19, impacting nearly half of all hospitalized patients, and is associated with high mortality, especially among those requiring renal replacement therapy [4–6]. We have recently shown that AKI may be driven in COVID-19 by a secondary thrombotic microangiopathy (TMA) phenomenon, as evidenced by low ADAMTS13 activity to von Willebrand factor (VWF:Ag) ratio [7]. However, the mechanism by which AKI occurs in COVID-19 has yet to be fully elucidated.

With the high frequency of AKI and thromboses in patients with COVID-19, biomarkers of endothelial damage/activation-related biomarkers have become of interest. Angiopoietin-1 (Ang-1) is an angiogenic growth factor that promotes vessel maturation and survival by activation of the Tie2 receptor (Tie2) on endothelial cells [8]. Ang-1 is expressed by pericytes and vascular smooth muscle cells and can stabilize endothelial functions by reducing inflammation and apoptosis of endothelial cells [9]. On the contrary, Angiopoietin-2 (Ang-2) enhances endothelial inflammation and hyperpermeability as it can act as an antagonist to Ang-1 and Tie2 signaling [9, 10]. We hypothesized that elevated Ang-2 would be associated with an increased risk for developing severe COVID-19-related AKI during the course of infection.

In this prospective observational study, adults (≥ 18 years old) presenting to the University of Cincinnati Medical Center Emergency Department (ED) with respiratory symptoms at triage suggestive of COVID-19 and with positive reverse transcription-polymerase chain reaction (RT-PCR) test for COVID-19 via nasopharyngeal swab were enrolled. This study was approved by the University of Cincinnati institutional review board (IRB) and performed under a waiver of informed consent. Blood samples were collected via routine draws for clinical indications in the ED. Circulating levels of Ang-1 and Ang-2 were determined in EDTA plasma using an enzyme-linked immunosorbent assay following the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA) using a DS2 ELISA processing system (Dynex Technologies, Inc, Chantilly, Virginia, USA). Serum creatinine was measured using a kinetic alkaline picrate (modified Jaffe) method using either a Beckman Coulter
AU480 Chemistry Analyzer (Brea, California, USA) or a Beckman Coulter AU5822 Chemistry Analyzer (Brea, California, USA). Patients were monitored through hospitalization until discharge/death if admitted from the ED or for 30 days if discharged from the ED. The primary outcome of interest was the development of severe AKI, defined as Kidney Disease: Improving Global Outcomes (KDIGO) Stage 2 + 3 according to serum creatinine (SCr) criteria [11]. The secondary outcome was the need for renal replacement therapy (RRT). Ang-2 levels were correlated with white blood cell count (WBC), C-reactive protein (CRP), interleukin (IL) 6, 8, 10, tumor necrosis factor-alpha (TNF-α), plasminogen, fibrinogen, D-Dimer, ADAMTS13 activity, VWF:ag, myoglobin, plasma neutrophil gelatinase-associated lipocalin (NGAL), and serum cystatin C.

Analysis of data was carried out using R software (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). Categorical data were reported as frequencies (%), while continuous data were reported as the median and interquartile range (IQR). Comparison of baseline Ang-1 and Ang-2 levels, as well as other laboratory values between COVID-19 patients with and without severe AKI, was carried out using the Mann–Whitney U-test. Proportions were compared between groups using Fisher’s exact test. Logistic regression analysis was performed to estimate the effect of changes in Ang-1 and Ang-2 levels when adjusted for the presence of comorbidities, and variable selection was performed using the stepwise algorithm.

A total of 51 COVID-19 patients were included. The median age was 50.5 (IQR: 39.3–66.0) years, and 57.7% were males. Their comorbidities are shown in Table 1. A total of 12 (23.5%) COVID-19 patients developed severe AKI, 8 (66.6%) needing RRT, and 3 (25.0%) died. No significant differences were observed in Ang-1 levels (2904.1 [IQR: 737.5–5111.] vs. 2670.7 [IQR: 1321.6–4711.] pg/mL; p = 0.916) or Ang-2/Ang-1 ratio (0.45 [IQR: 0.07–1.08] vs. 1.15 [IQR: 0.53–2.47] pg/mL; p = 0.201) in those who developed severe AKI versus those who did not. Nonetheless, Ang-2 levels were found to be significantly higher in those who developed severe AKI (4715.7 [IQR: 2768.8–17,919.1] vs. 2462.4 [IQR: 1699.0–3641.8] pg/mL; p = 0.047) (Fig. 1a). Moreover, Ang-2 level was the highest in those who required RRT (13,372.7 [IQR: 3604.4–20000] vs. 2556.1 [IQR: 1699–3235] pg/mL; p = 0.037) (Fig. 1b).

Table 1 Baseline demographics of the Cincinnati emergency department COVID-19 cohort

| Variable | All patients (n = 51) | KDIGO AKI stage | p-value |
|----------|----------------------|-----------------|---------|
|          |                      | 0 + 1           | 2 + 3   |
| Age (years): median (IQR) | 50.5 (41–66) | 47 (37.5–64.0) | 66 (56.5–70.2) | 0.005 |
| Sex (male): n (%) | 30 | 23 (76.7%) | 7 (23.3%) | 1.000 |
| BMI: median (IQR) | 28.5 (24.8–33.5) | 29.5 (25.8–34.5) | 24.5 (21.6–27.5) | 0.018 |
| Race: n (%) | Black | 21 | 12 (57.1%) | 9 (42.9%) | 0.036 |
|          | Hispanic | 18 | 17 (94.4%) | 1 (5.6%) | 0.036 |
|          | White | 9 | 7 (77.8%) | 2 (22.2%) | 0.036 |
|          | Other | 3 | 3 (100%) | 0 (0%) | 0.036 |
| Comorbidities: n (%) | Coronary artery disease | 8 | 3 (37.5%) | 5 (62.5%) | 0.012 |
|          | Heart failure | 9 | 3 (33.3%) | 6 (66.7%) | 0.003 |
|          | Hypertension | 26 | 15 (57.7%) | 11 (42.3%) | 0.002 |
|          | Hyperlipidemia | 15 | 11 (73.3%) | 4 (26.7%) | 0.730 |
|          | Diabetes | 21 | 15 (71.4%) | 6 (28.6%) | 0.738 |
|          | Chronic obstructive pulmonary disease | 8 | 4 (50%) | 4 (50%) | 0.076 |
|          | Asthma | 8 | 6 (75%) | 2 (25%) | 1.000 |
|          | Chronic kidney disease | 6 | 1 (16.7%) | 5 (83.3%) | 0.002 |
|          | Chronic liver disease | 7 | 3 (42.9%) | 4 (57.1%) | 0.044 |
|          | Cerebrovascular disease | 1 | 0 (0%) | 1 (100%) | 0.375 |
|          | Cancer | 4 | 1 (25%) | 3 (75%) | 0.036 |
|          | Acquired immunodeficiency (HIV, transplant) | 3 | 2 (66.7%) | 1 (33.3%) | 1.000 |
|          | Autoimmune disease | 2 | 2 (100%) | 0 (0%) | 1.000 |

*BMI Body Mass Index, KDIGO Kidney Disease: Improving Global Outcomes, AKI Acute Kidney Injury

p < 0.05

 Springer
Ang-2 was found to be positively correlated with WBC ($r = 0.596; p < 0.001$), IL-6 ($r = 0.280; p = 0.049$), TNF-α ($r = 0.316; p = 0.024$), fibrinogen ($r = 0.405; p = 0.009$), D-dimer ($r = 0.552; p = 0.008$), cystatin C ($r = 0.345, p = 0.019$), NGAL ($r = 0.431, p = 0.002$), and negatively correlated with plasminogen ($r = −0.370; p = 0.007$) and ADAMTS13 ($r = −0.302; p = 0.031$). No correlation was observed for IL-10 ($p = 0.794$), CRP ($p = 0.11$), or VWF:ag ($p = 0.427$).

In multivariate logistic regression, both pre-existing chronic kidney disease and hypertension were significantly associated with increased odds of severe AKI, with adjusted odds ratios (ORs) of 31.8 (95% CI 1.18–854.88) and 22.0 (95% CI 1.15–420.32), respectively. An increase in 1000 pg/mL of Ang-2 was associated with a 39% increase in odds of severe AKI (OR $1.39 [95\% CI 1.05–1.86]$). Full results are presented in Supplemental Table 1.

In this prospective study, we observed that Ang-2 levels measured at ED presentation are significantly increased in patients at risk of developing severe AKI. Moreover, we observed that elevated Ang-2 is an independent predictor of severe AKI and RRT. Our findings are in agreement with Smadja et al. [12], who reported significantly higher levels of Ang-2 in intensive care unit-admitted COVID-19 patients. They observed that patients with Ang-2 levels greater than 5000 pg/mL had ninefold higher odds of ICU admission. Our findings are also in agreement with Araujo et al. [13] who observed that elevated Ang-2 levels were significantly associated with increased odds of severe AKI and need for RRT in ICU-admitted non-COVID-19 acute respiratory distress syndrome (ARDS) patients.

Overall, our results are consistent with a picture of endothelial injury and a thrombotic microangiopathy phenomenon in COVID-19-associated AKI, further supported by the negative correlation with ADAMTS13 activity and positive correlations with fibrinogen and D-dimer. These results are consistent with elevations of Ang-2 observed in other forms of TMA [14–16]. Ang-2 was also correlated with several pro-inflammatory biomarkers, consistent with a hyperinflammatory response that can produce endothelial injury. Endothelium activation can lead to the release of Ang-2 from Weibel–Palade (WP) bodies [17]. Interestingly, however, we did not observe significant correlation between Ang-2 and VWF:ag ($p = 0.427$). Philippe et al. [18] reported observing two distinct biomarker profiles, with VWF:ag increased in accordance with disease severity, while Ang-2 was elevated only in the critically ill. Taken together, this suggests that endothelial VWF secretion in COVID-19 may in part occur via pathways different than that of Ang-2. Indeed, while VWF is also secreted via WP bodies in the basal and regulated secretory pathways, the endothelium may also directly secrete VWF via a constitutive secretory pathway using small anterograde carriers [19]. Moreover, COVID-19 is associated with platelet hyperactivity, which occurs via multiple mechanisms, including spike protein binding to platelet angiotensin-converting enzyme 2 (ACE2) receptors, resulting in platelet activation and alpha granule release, which contains VWF in high molecular weight forms [20].

Ang-2 inhibits the protective anti-inflammatory Ang-1/Tie2 signaling cascade [17]. The Tie2 receptor is a central regulator in protecting the vasculature against thrombus formation in the setting of systemic inflammation, such as that seen in sepsis [21]. In a pilot study of critically ill patients with TMA and anti-glomerular basement membrane disease, plasma exchange was shown to be an effective method to remove excess circulating Ang-2, returning to almost normal values with $\leq 4$ treatments [17]. As such, the investigation of the pathophysiologic role of Ang-2 in COVID-19 should be prioritized as targeting Ang-2 via plasma exchange or other inhibitory approaches are potential therapies in patients with
severe COVID-19. Future longitudinal studies are needed to fully elucidate the role of Ang-2 in COVID-19 endothelial dysfunction and multiorgan injury and the specificity of Ang-2 for COVID-19 AKI.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10456-021-09782-w.

**Funding** This study was funded by the University of Cincinnati College of Medicine Special Coronavirus (COVID-19) Research Pilot Grant Program and the Lymphatic Malformation Institute.

**Declarations**

**Conflict of interest** The authors do not have any conflicts of interest concerning this publication.

**References**

1. Bradley BT, Maioli H, Johnston R et al (2020) Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. The Lancet 396:320–332
2. Varga Z, Flammer AJ, Steiger P et al (2020) Endothelial cell infection and endotheliitis in COVID-19. Lancet 395:1417–1418
3. Pons S, Fedil S, Azoulay E et al (2020) The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. Crit Care 24:353
4. Gupta S, Coca SG, Chan L, et al. (2020) AKI Treated with Renal Replacement Therapy in Critically Ill Patients with COVID-19. JASN [Internet]. [cited 2020 Nov 11]; Available from: https://jasn.asnjournals.org/content/early/2020/09/02/ASN.2020050615.
5. Chan L, Chaudhary K, Saha A, et al. (2020) AKI in Hospitalized Patients with COVID-19. JASN [Internet]. [cited 2020 Nov 11]; Available from: https://jasn.asnjournals.org/content/early/2020/09/02/ASN.2020050615.
6. Cheruiyot I, Henry B, Lippi G, et al. Acute Kidney Injury is Associated with Worse Prognosis In COVID-19 Patients: A Systematic Review and Meta-analysis. 1. 2020;91:ahead of print-ahead of print.
7. Henry BM, Benoit SW, de Oliveira MHS et al (2020) ADAMTS13 activity to von Willebrand factor antigen ratio predicts acute kidney injury in patients with COVID-19: Evidence of SARS-CoV-2 induced secondary thrombotic microangiopathy. Int J Lab Hematol. https://doi.org/10.1111/ijlh.13415
8. Yancopoulos GD, Davis S, Gale NW et al (2000) Vascular-specific growth factors and blood vessel formation. Nature 407:242–248
9. Neuhäuss A-K, Gutbier B, Friedemann T, et al. Angiopoietins: Possible biomarkers in severe pneumonia? European Respiratory Journal [Internet]. 2012 [cited 2020 Nov 5];40. Available from: https://erj.ersjournals.com/content/40/Suppl_56/P830.
10. Thurston G, Daly C (2012) The complex role of angiopoietin-2 in the angiopoietin-tie signaling pathway. Cold Spring Harb Perspect Med 2:a006550
11. KDIGO AKI Working Group (2012) KDIGO clinical practice guideline for acute kidney injury. Kidney International Suppl 2:1
12. Smadja DM, Guerin CL, Chocron R et al (2020) Angiopoietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients. Angiogenesis 23(4):611–620
13. Araújo CB, de Oliveira Neves FM, de Freitas DF et al (2019) Angiopoietin-2 as a predictor of acute kidney injury in critically ill patients and association with ARDS. Respirology 24:345–351
14. Lukasz A, Bencej Thamm K, et al. Involvement of Angiopoietin-2 and Tie2 Receptor Phosphorylation in STEC-HUS Mediated by Escherichia coli O104:H4 [Internet]. Mediators of Inflammation. Hindawi; 2015 [cited 2020 Dec 29]. p. e670248. Available from: https://www.hindawi.com/journals/mi/2015/670248/.
15. Ueda N, Chihara D, Kohno A et al (2014) Predictive Value of Circulating Angiopoietin-2 for Endothelial Damage-Related Complications in Allogeneic Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant 20:1335–1340
16. Shimizu M, Inoue N, Kuroda M et al (2017) Angiopoietin-1 and -2 as markers for disease severity in hemolytic uremic syndrome induced by enterohemorrhagic Escherichia coli. Clin Exp Nephrol 21:76–82
17. Lovric S, Lukasz A, Hafer C et al (2010) Removal of elevated circulating angiopoietin-2 by plasma exchange—a pilot study in critically ill patients with thrombotic microangiopathy and anti-glomerular basement membrane disease. Thromb Haemost 104:1038–1043
18. Philippe A, Chocron R, Gendron N et al (2021) Circulating Von Willebrand factor and high molecular weight multimers as markers of endothelial injury predict COVID-19 in-hospital mortality. Angiogenesis. https://doi.org/10.1007/s10456-020-09762-6
19. Lopes da Silva M, Cutler DF (2016) von Willebrand factor multimerization and the polarity of secretory pathways in endothelial cells. Blood 128:277–285
20. Zhang S, Liu Y, Wang X et al (2020) SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 13:120
21. Higgins SJ, De Ceunynck K, Kellum JA et al (2018) Tie2 protects the vasculature against thrombus formation in systemic inflammation. J Clin Invest. 128:1471–1484

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.