LETTER

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

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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

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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%)	
White	9	7 (77.8%)	2 (22.2%)	
Other	3	3 (100%)	0 (0%)	
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

$p < 0.05$
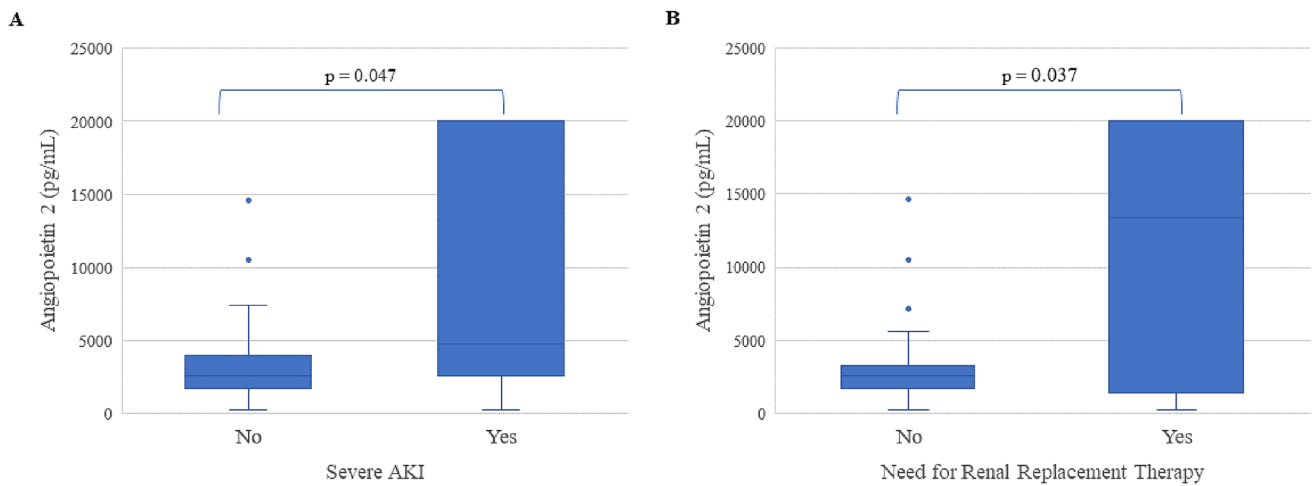
Fig. 1 Angiopoietin-2 levels in patients developing severe AKI (**a**) and in patients requiring renal replacement therapy (RRT) (**b**)

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. 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 ≤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.

Declarations

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

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