Acute Kidney Injury in COVID-19: Emerging Evidence of a Distinct Pathophysiology

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The most common reported reasons for intensive care unit admission for patients with severe coronavirus disease 2019 (COVID-19) are either hypoxemic respiratory failure leading to mechanical ventilation or hypotension requiring vasopressor support. Data on AKI are either lacking[1] or only reporting incidence on the basis of case series and retrospective studies.[2] In this Perspective, we emphasize that AKI can be a severe complication of COVID-19 and highlight the importance of assessing, defining, and reporting the course of AKI.

Understandably relevant information that normally would be part of clinical descriptions and research publications has not been collected because of the magnitude and accelerated pace of the COVID-19 pandemic. Of great relevance is a preprint in *medRxiv* reporting a 23% AKI incidence among 85 patients (over 60% in high-risk patients). The authors analyzed kidney histology from autopsies of six patients who had AKI showing severe acute tubular necrosis with lymphocyte and macrophage infiltration, but it is not clear from this report if these patients had actually developed cortical necrosis (B. Diao, C.H. Wang, R.S. Wang, Z.Q. Feng, Y.J. Tan,

H.M. Wang, *et al.*: Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] infection [preprint posted online April 10, 2020]. *medRxiv* doi:10.1101/2020.03.04.20031120). An important report on autopsy findings from deceased patients with COVID-19 again demonstrated prominent acute proximal tubular injury, but also peritubular erythrocyte aggregation and glomerular fibrin thrombi with ischemic collapse.[3] This paper also reported endothelial damage, hemosiderin deposition, pigment casts related to rhabdomyolysis, and inflammation. Notably, some of these patients lacked evidence of AKI as detected by routine measures (creatinine and/or BUN), highlighting the possibility of substantial subclinical kidney injury.

Recent clinical and autopsy reports of COVID-19 from China and the United States confirm increased clotting and disseminated intravascular coagulation with small vessel thrombosis and pulmonary infarction.[4] Further, elevated d-dimer and low platelet levels correlated with worse outcomes.[4] We are aware that some patients with COVID-19 manifest evidence of microangiopathy in other organ systems, such as splenic infarction or presenting
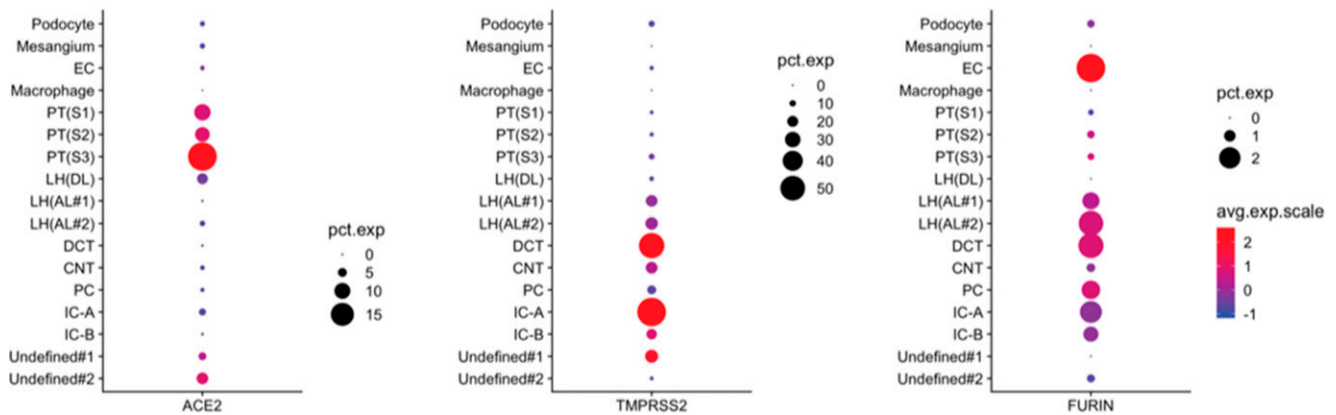
symptoms of loin pain and hematuria suggesting renal infarction. Numerous observations by treating physicians attest that there is increased occurrence of circuit clotting in patients with COVID-19 undergoing dialysis. COVID-19 is also associated with increased myocardial injury that mimics myocardial infarction, possibly from myocarditis and microangiopathy.[5] Thus, it is conceivable that the hypercoagulable state that appears to be a characteristic complication of severe

Figure 1. Single-cell RNA of SARS-COV-2 receptor (ACE2) and known priming proteases in the kidney. There is no clear correspondence in single-cell RNA between ACE2 and TMPRSS2. EC, endothelial cell; PT, proximal tubule (S1, Segment one; S2 Segment two, S3 Segment three); LH(DL), loop of henle(descending thin limb); LH(AL), loop of henle(descending limb type one [#type 1] and type two [type #2]); DCT, distal convoluted tubule; CNT, connecting tubule; PC, principal cell; IC-A, alpha intercalated cell; IC-B, beta intercalated cell; pct. exp, percentexpressed. Data were extracted with permission from human kidney single-cell RNA-sequencing data.[12,13]

COVID-19 could, in some cases, foster the evolution of acute tubular necrosis to cortical necrosis and, therefore, irreversible kidney failure.

Innate immunity and coagulation pathways are intricately linked.[6] COVID-19–associated macrophage activation, hyperferritinemia, cytokine storm, and release of pathogen-associated molecular patterns and damage-associated molecular proteins can result in release of tissue factor and activation of coagulation factors that create a predisposition to hypercoagulability.[6] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may also target lymphocytes as they express angiotensin-converting enzyme 2 (ACE2), leading to lymphocyte activation and, consequently, activation-induced cell death than can result in lymphopenia of both CD4^{+} and CD8^{+} T cells.[7] Further, procoagulation pathways and complement systems can activate each other. In support of this interaction in COVID-19, Diao and colleagues (B. Diao, C.H. Wang, R.S. Wang, Z.Q. Feng, Y.J. Tan, H.M. Wang, *et al.*: Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] infection [preprint posted online April 10, 2020]. *medRxiv* doi:10.1101/2020.03.04.20031120) observed strong complement C5b-9 (membrane attack complex)

deposition in renal tubules of six patients with SARS-CoV-2 infection, suggesting activation of the complement pathway. An interaction between angiotensin II (AngII) overactivity, innate/adaptive immune and complement pathways, and the coagulation system could influence AKI severity and outcomes. Inflammation-induced erythrocyte aggregation (reflected as elevated erythrocyte sedimentation rate) and heme-mediated pathology may worsen oxidative stress, inflammation, and complement activation, to aggravate microvascular injury.[8] Further, organ crosstalk between the injured lung, the heart, and the kidney can worsen pathology. Detailed studies to decipher the nature of coagulation dysfunction, microangiopathy, and potential role for innate immune and complement pathways are required to gain further insights regarding kidney pathology in COVID-19.

Also of interest is the finding that SARS-CoV-2 nucleocapsid protein was observed in tubular structures in the kidneys from the six patients examined, and nucleocapsid protein–positive inclusion bodies were also observed in the cytoplasm (B. Diao, C.H. Wang, R.S. Wang, Z.Q. Feng, Y.J. Tan, H.M. Wang, *et al.*: Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] infection [preprint posted online April 10, 2020]. *medRxiv*

doi:10.1101/2020.03.04.20031120). Su *et al.*[3] similarly observed the presence of virus-like particles in podocytes and renal tubular epithelial cells by electron microscopy, and SARS-CoV-2 nucleoprotein antibody stained renal tubular epithelia positive, but the specificity of the antibody used needs to be established. Although, as far as we know, SARS-CoV-2 RNA, has not been detected in the kidney, these results indicate that SARS-CoV-2 could directly infect human kidney tubules and induce cytoplasmic renal tubular inclusions, a feature observed in other virus-associated nephropathies. Thus, although AKI may be attributable to hypotension and decreased kidney perfusion secondary to hemodynamic or hemostatic factors or associated sepsis, one needs to consider that viral infection of the kidneys with viral replication directly in kidney parenchyma also plays a role.

The main binding site for SARS-CoV-2, like SARS-CoV, is the ACE2 protein, which is expressed in the kidney much more than the lungs.[9,10] ACE2 is expressed on the brush border apical membrane of the proximal tubule, where it colocalizes with angiotensin-converting enzyme (ACE), and is also present at lower levels in podocytes.[10] It is conceivable that the virus could enter the kidney by invading podocytes

first, and thus gain access to the tubular fluid and subsequently bind to ACE2 in the proximal tubule. In primary human airway epithelia, ACE2 is expressed apically, and SARS-CoV-2 infection predominantly occurs on the apical surface, but infection can occur on the basolateral surface at low efficiency.[11] Coronavirus entry into host target cells also requires fusion of the viral envelope with cellular membranes. Fusion-activated SARS-CoV peptides are created by specific proteolytic cleavage of the S proteins, in a step called "priming." As a consequence, cell infectivity not only depends on ACE2 expression, but is also governed by types of proteases found in a given cell type. In the kidney, Transmembrane protease, serine 2 (TMPRSS2)[12–14] (Figure 1), which primes the SARS-CoV-2 S protein, is robustly expressed in the distal nephron rather than the proximal tubule. It remains to be determined if other TMPRSS in the proximal tubule can mediate the priming step, such as TMPRSS 4, 5, or 9. Alternatively, tropism of SARS-CoV-2 might be expanded by the unique

furin cleavage site in the Spike protein that is processed during biogenesis.[14]

Any effect of proteinuria, hyperinflammation, or tubular injury on proximal tubular ACE2 expression or SARS-CoV-2 viral entry is currently unknown. Viral replication in podocytes and the ensuing damage could in theory account for the proteinuria that has been reported in patients with COVID-19.[2] Further, COVID-19–associated hemophagocytic macrophage activation and microangiopathy could also cause AKI and podocyte damage. Of interest, cases of COVID-19–associated collapsing glomerulopathy have been described.[15]

Regardless of direct viral infection of the kidney, AngII is likely increased in the context of acute lung injury[16] and there is evidence that ACE2 is downregulated in AKI. This may lead to type 1 angiotensin receptor activation as well as decreased angiotensin (1–7) formation and subsequent worsening of AKI. This is particularly important in subpopulations of patients who have CKD, especially those with diabetic kidney disease (DKD). ACE2 and ACE mRNA and

protein expression are altered in mouse models of DKD and in patients with DKD.[10,17] Thus, patients with CKD, especially those with DKD, who develop COVID-19 may be at higher risk of AKI because of baseline upregulation of the ACE and downregulation of ACE2, a combination that primes a proinflammatory (including complement activation) and profibrotic state in the kidneys.

Interestingly, a recent study described single-cell transcriptome analysis in 15 normal human kidney samples.[18] In this study, the proportions of kidney cells expressing ACE2, the SARS-CoV-2 binding site, and proteases of the TMPRSS family were compared between occidental and Asian individuals.[18] Interestingly, the expression of ACE2 and kidney disease–related genes was higher in occidental donors relative to Asian donors. This would suggest that the susceptibility to kidney injury from coronavirus infection might be higher in individuals of occidental rather than Asian descent. We are not aware, however, of data supporting this possibility.

Despite the very limited information on kidney involvement in COVID-19, AKI appears to involve a complex process driven by virus-mediated injury, cytokine storm, AngII pathway activation, dysregulation of complement, hypercoagulation, and microangiopathy interacting with common and known risk factors for AKI (Figure 2). There is paucity of data regarding clinical and laboratory characteristics of AKI in patients with COVID-19. We urge that further studies describing and analyzing the clinical course of patients with COVID-19 include appropriate indices of kidney function and diagnosis of AKI in their analyses, including kidney injury markers, urine microscopy, quantified urine protein, urine output, and urine electrolytes. Markers of macrophage activation, coagulation, microangiopathy, and complement activation, as well as kidney imaging and need for KRT (with relevant details), are important data needed to further our understanding of AKI pathophysiology associated with COVID-19. Rates of reversibility of, or partial improvement in, kidney function and any
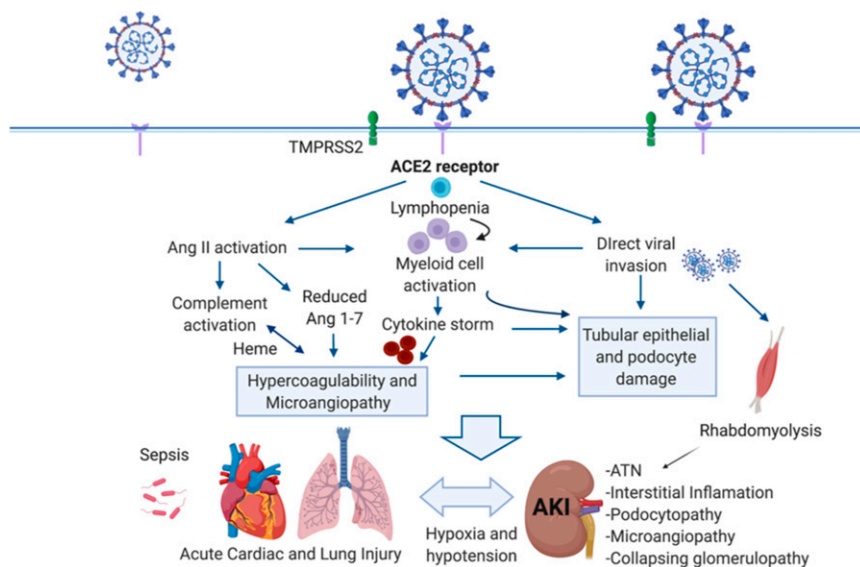
Figure 2. Targeting of ACE2 by SARS-CoV-2 results in angiotensin dysregulation, innate and adaptive immune pathway activation, and hypercoagulation to result in organ injury and AKI associated with COVID-19. Organ crosstalk between the injured lungs, the heart, and the kidney may further propagate injury. CD8+ T-cells and natural killer cells can restrain macrophage activation and are potential targets for SARS-CoV-2. Ang 1–7, angiotensin 1–7; ATN, acute tubular necrosis. ACE2, angiotensin converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease, serine 2.

kidney biopsy results (including immu-nofluorescence and electron micros-copy) should be reported. In the rush to report medical complications of COVID-19, we are missing valuable clinical information. Speculation about specific interventions would not be ap-propriate until we obtain appropriate in-formation. We advocate for a complete and standardized appraisal of the clinical and laboratory picture so that preventa-tive and therapeutic strategies for AKI can be appropriately designed and implemented.

REFERENCES

1. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al.: Covid-19 in critically ill patients in the seattle region - case series [published online ahead of print Mar 30, 2020]. *N Engl J Med* doi:10.1056/NEJMoa2004500

2. Cheng Y, Luo R, Wang K, Zhang M, Wang Z, Dong L, et al.: Kidney disease is associated with in-hospital death of patients with COVID-19 [published online ahead of print Mar 20, 2020]. *Kidney Int* doi:10.1016/j.kint.2020.03.005

3. Su H, Yang M, Wan C, Yi LX, Tang F, Zhu HY, et al.: Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China [published online ahead of print Apr 9, 2020]. *Kidney International* doi:10.1016/j.kint.2020.04.003

4. Tang N, Li D, Wang X, Sun Z: Abnormal co-agulation parameters are associated with poor prognosis in patients with novel coro-navirus pneumonia. *J Thromb Haemost* 18: 844–847, 2020

5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al.: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* 395: 1054–1062, 2020

6. Delvaeye M, Conway EM: Coagulation and innate immune responses: Can we view them separately? *Blood* 114: 2367–2374, 2009

7. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al.: Clinical and immunological features of severe and moderate coronavirus disease 2019 [published online ahead of print Apr 13, 2020]. *J Clin Invest* doi:10.1172/JCI137244

8. Frimat M, Tabarin F, Dimitrov JD, Poitou C, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, et al.: Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. *Blood* 122: 282–292, 2013

9. Serfozo P, Wysocki J, Gulua G, Schulze A, Ye M, Liu P, et al.: Ang ii (angiotensin ii) conversion to angiotensin-(1-7) in the circulation is pop (prolyloligopeptidase)-dependent and ace2 (angiotensin-converting enzyme 2)-independent. *Hypertension* 75: 173–182, 2020

10. Ye M, Wysocki J, William J, Soler MJ, Cokic I, Batlle D: Glomerular localization and ex-pression of angiotensin-converting enzyme 2 and angiotensin-converting enzyme: Impli-cations for albuminuria in diabetes. *J Am Soc Nephrol* 17: 3067–3075, 2006

11. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al.: ACE2 receptor expression and severe acute respiratory syndrome co-ronavirus infection depend on differentiation of human airway epithelia. *J Virol* 79: 14614–14621, 2005

12. Wu H, Uchimura K, Donnelly EL, Kirita Y, Morris SA, Humphreys BD: Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. *Cell Stem Cell* 23: 869–881.e8, 2018

13. Wilson PC, Wu H, Kirita Y, Uchimura K, Ledru N, Rennke HG, et al.: The single-cell tran-scriptomic landscape of early human di-abetic nephropathy. *Proc Natl Acad Sci U S A* 116: 19619–19625, 2019

14. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D: Structure, function, and antigenicity of the SARS-CoV-2 Spike glycoprotein. *Cell* 181: 281–292.e6, 2020

15. Larsen CP, Bourne TD, Wilson JD, Saqqa O, Sharshir MA: Collapsing glomerulopathy in a patient with coronavirus disease 2019 (COVID-19) [published online ahead of print Apr 9, 2020]. *Kidney Int Rep* doi:10.1016/j.ekir.2020.04.002

16. Reddy R, Asante I, Liu S, Parikh P, Liebler J, Borok Z, et al.: Circulating angiotensin pep-tides levels in acute respiratory distress syn-drome correlate with clinical outcomes: A pilot study. *PLoS One* 14: e0213096, 2019

17. Mizuiri S, Hemmi H, Arita M, Ohashi Y, Tanaka Y, Miyagi M, et al.: Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. *Am J Kidney Dis* 51: 613–623, 2008

18. Pan XW, Xu D, Zhang H, Zhou W, Wang LH, Cui XG: Identification of a potential mecha-nism of acute kidney injury during the COVID-19 outbreak: A study based on single-cell transcriptome analysis [published online ahead of print Mar 31, 2020]. *Intensive Care Med* doi:10.1007/s00134-020-06026-1