Clinical Kidney Journal

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LEADING EUROPEAN NEPHROLOGY

Clinical Kidney Journal, 2020, vol. 13, no. 6, 1101–1104

doi: 10.1093/ckj/sfaa209
Exceptional Case

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Severe acute respiratory syndrome coronavirus 2 indirectly damages kidney structures

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ABSTRACT

Background. The objectives were to characterize Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) in patients with acute kidney injury (AKI).

Methods. Kidney biopsy samples in two Caucasian patients and one African with COVID-19 AKI were investigated.

Results. All patients had a high-level non-selective glomerular proteinuria. SARS-CoV-2 samples by real-time polymerase chain reaction (RT- PCR) assay were all-negative, as well as for virus particles in the kidney by electron microscopy. The three patients and patients with other AKI did not differ significantly with regard to angiotensin-converting enzyme 2 and transmembrane protease serine 2 kidney staining.

Conclusions. The kidney damage particularly in Caucasians in COVID-19 seems to be an AKI, possibly by the systemic inflammatory response.

Keywords: angiotensin-converting enzyme 2, Caucasian patient, COVID-19, electron microscopy, kidney disease, SARS-CoV-2, tubular proteinuria

Received: 15.9.2020; Editorial decision: 28.9.2020

INTRODUCTION

Acute kidney injury (AKI) occurs in >10% of patients with coronavirus disease 2019 (COVID-19) and is associated with increased mortality. Although collapsing glomerulopathy has recently been described in African patients with an Apolipoprotein L1 (*APOL1*) high-risk genotype, the incidence of glomerular impairment among Caucasian patients with COVID-19 has not been determined.

We now know that Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets epithelial cells: the viral structural

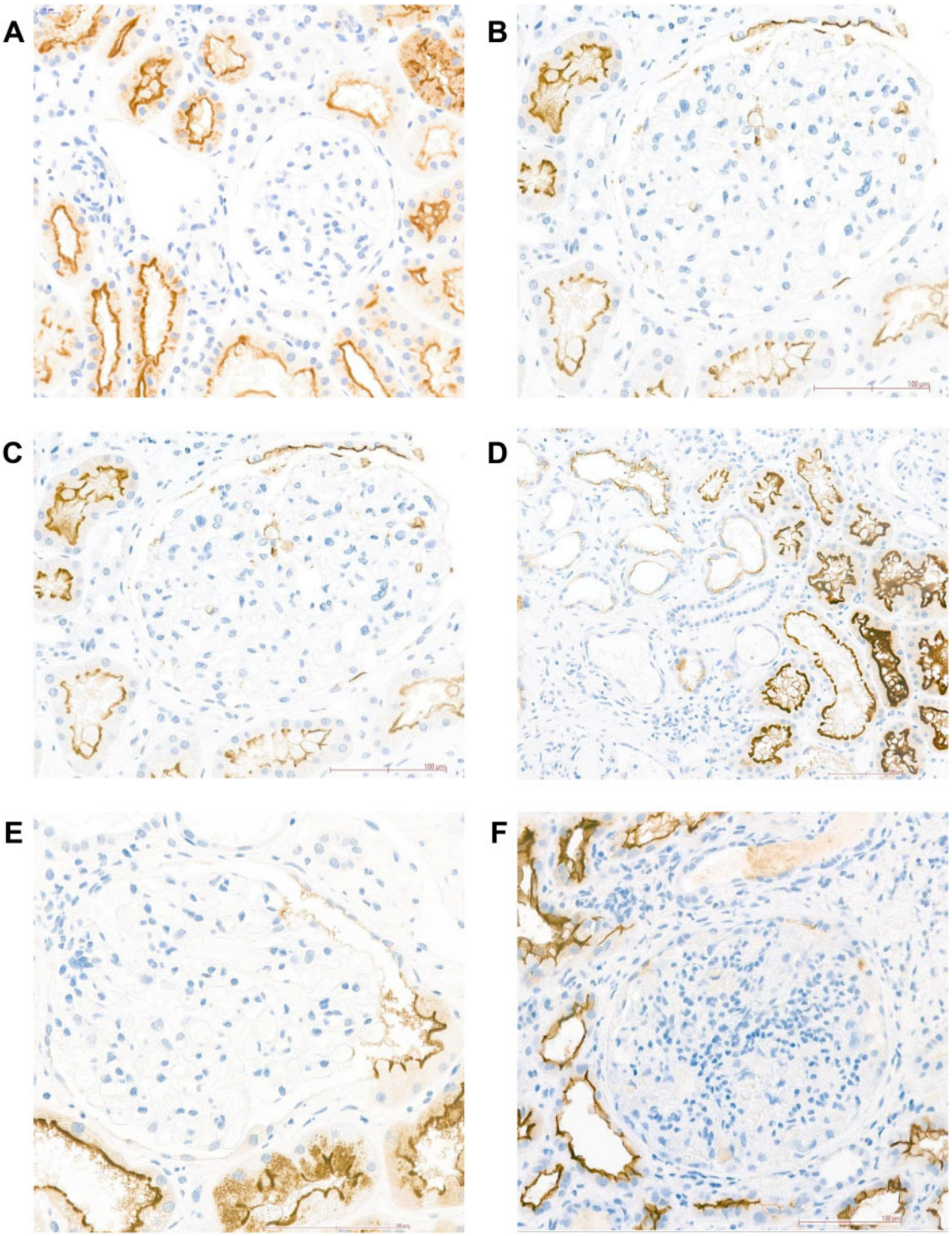

FIGURE 1: Representative ACE2 staining in patients with COVID-19, healthy individuals and patients with non-COVID-19-associated kidney diseases. (**A**) Strong staining in the brush border of proximal tubules and no staining in the glomerulus in a normal kidney (magnification: ×200). (**B** and **C**) Enhanced staining in PECs from a Caucasian patient with COVID-19 (**B**) and in an ethnic African patient with COVID-19 and collapsing glomerulopathy (**C**) (×400). (**D**) Diabetic nephropathy: faint cytoplasmic staining in damaged tubules (×100). (**E** and **F**) Weak staining in the PECs in cases of ATN (**E**) and focal segmental glomerulosclerosis (**F**) not associated with COVID-19 nephropathy (×400).

Clinical Kidney Journal

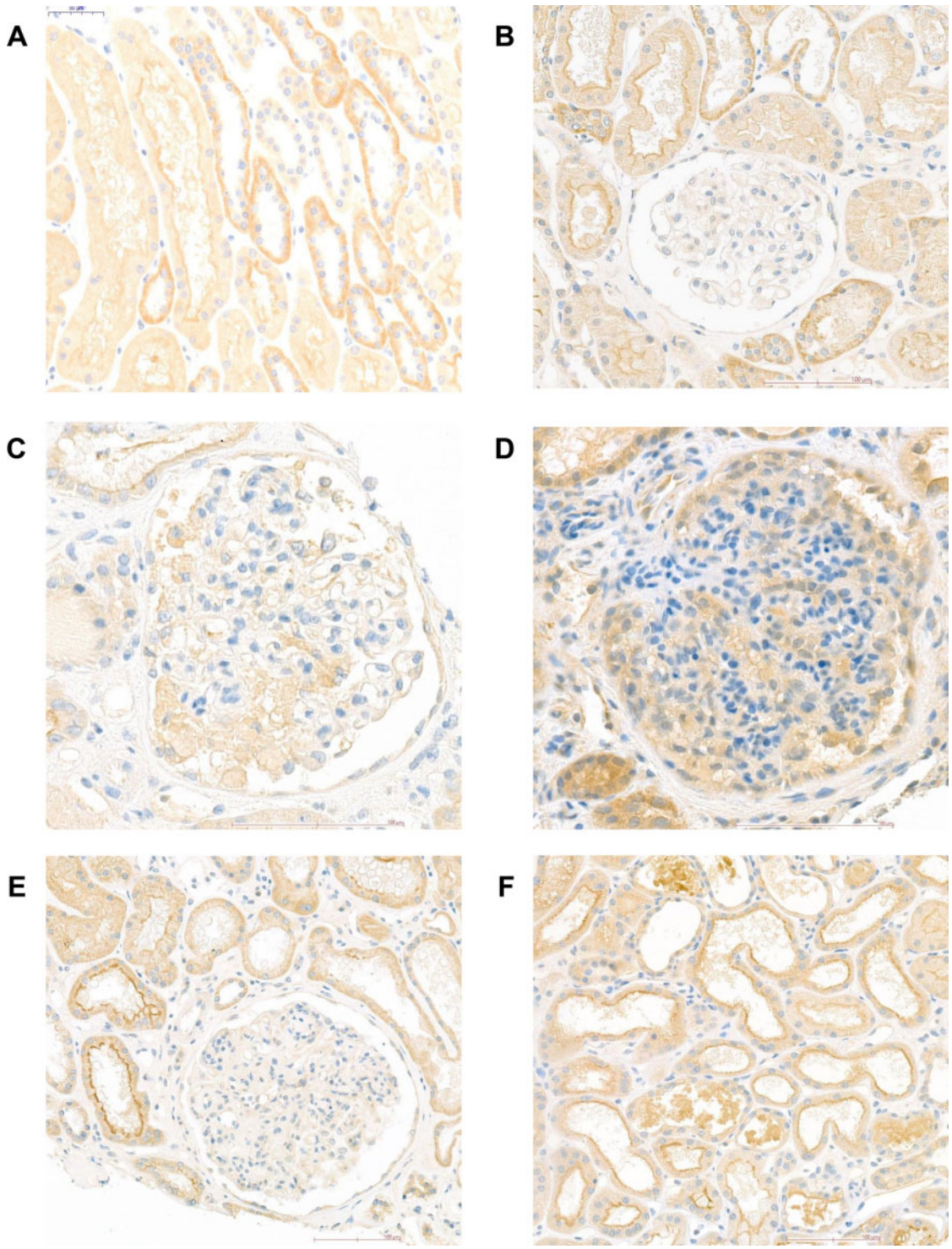

FIGURE 2: Representative TMPRSS2 staining in patients with COVID-19, healthy individuals and patients with non-COVID-19-associated kidney diseases. (**A**) Strong cytoplasmic staining of distal and proximal tubules (magnification: ×300). (**B**) Enhanced staining in the brush border of proximal tubules and no glomerulus staining in a Caucasian patient with COVID-19 (×400). (**C**) Diffuse, faint staining of podocytes in an ethnic African patient with COVID-19 and collapsing FSGS (×400). (**D**) In non-COVID patients with FSGS, the podocyte staining pattern was similar to that seen in (**C**) (×400). (**E** and **F**) Enhanced staining in the brush border of proximal tubules in a patient with diabetic nephropathy (**E**) and a patient with ATN (×200) (**F**). No staining in the glomerular tuft was observed in the patient with diabetic nephropathy.

spike protein binds to the angiotensin-converting enzyme 2 (ACE2) as an entry receptor and is then primed by the cellular transmembrane protease serine 2 (TMPRSS2) [1]. The mechanisms underlying renal injury in COVID-19 (direct viral toxicity and/or secondary cytokine-mediated damage) are subject to debate.

Hence, we sought to determine (i) the characteristics of kidney damage in Caucasian patients with COVID-19 and (ii) the potential pathogenic mechanisms of SARS-CoV-2 infection in this context. Furthermore, we used immunohistochemical techniques to assess and compare the ACE2 and TMPRSS2 staining

patterns in COVID-19-associated AKI, AKI with other aetiologies and other kidney diseases.

MATERIALS AND METHODS

Please see supplementary file.

RESULTS

Clinical and laboratory findings on admission

Disease course and treatment and pathological assessment are presented in detail in the supplementary file.

Expression of ACE2 (Figure 1)

In samples from healthy kidney, strong ACE2 staining was observed in the apical brush border in proximal convoluted tubules (PCT)s but not in podocytes, parietal epithelial cells (PECs), mesangial cells and endothelial cells. In samples from the patients with COVID-19, the PECs were moderately positive for ACE2. The podocytes were seen to express ACE2 in the two Caucasian cases but not in the ethnic African patient with Focal segmental glomerulosclerosis (FSGS). ACE2 expression by podocytes and PECs was less intense in control patients with glomerular damage or ATI not related to COVID-19 than in the patients with COVID-19.

In all samples (regardless so the diagnosis), ACE2 staining was less intense in damaged tubules than in undamaged tubules. The immunohistochemical data are summarized in Supplementary data, Table S3a.

Expression of TMPRSS2 (Figure 2)

In normal kidney samples, the endothelial cells, Distal convoluted tubules (DCT)s and PCTs were positive for TMPRSS2. Of the three patients with COVID-19, only the ethnic African patient with FSGS showed moderately intense TMPRSS2 staining in the podocytes. The level of expression was similar to that observed in non-COVID-19 patients with Focal segmental glomerulosclerosis (FSGS). The DTCs and PCTs from all the other controls and patients (including Patients #2 and #3) were negative for TMPRSS2. The PECs showed no staining ($n = 3$), faint staining ($n = 13$) and staining moderate ($n = 3$). There were no differences between the three COVID-19 cases and the various non-COVID-19 categories. In cases of Acute tubular necrosis (ATN) (regardless of the cause), epithelial cells from the proximal tubules showed moderate apical staining and enhanced expression in the brush border. Immunohistochemical data are summarized in Supplementary data, Table S3b.

DISCUSSION

Please also see the detailed discussion in the supplementary file.

Recent studies suggest that SARS-CoV-2 enters host cells by binding to ACE2 receptors and TMPRSS2 (a co-receptor located on the cell surface of many cells, including the renal tubular epithelium). In normal kidney, only epithelial tubular cells and podocytes express ACE2. An infected cell might modulate ACE2 expression to allow viral entry [2], and one would expect to see less intense staining. However, we observed no differences between normal kidney, patients with COVID-19, and other causes of ATN with regard to ACE2 staining in undamaged PCTs. This result suggests that even though ATN is the most frequently described lesion [3], SARS-CoV-2 infection does not seem to affect the PCTs directly or modify ACE2 expression. We also noted marked ACE2 expression in PECs, and no co-localization with TMPRSS2. This body of indirect evidence suggests that the kidneys had not been infected with SARS-CoV-2. A study found that levels of TMPRSS2 expression (according to an real-time polymerase chain reaction (RT- PCR) assay) were very low in podocytes and PCTs [4]. In our study, TMPRSS2 and ACE2 staining was intense in normal PCTs but reduced in damaged tubule cells, in lesions related or non-related to SARS-CoV-2. In contrast, Kuba *et al*. reported that the SARS-CoV-2 spike protein downregulated the expression of ACE2 [5]. We hypothesize that the low level of ACE2 staining seen in damaged tubule cells was due to the loss of brush border following apoptosis or cell dedifferentiation, as a mechanism for cell protection. Given that ACE2 is known to have anti-oxidant activity, the lack of this enzyme might also accentuate the severity of the lesions.

To conclude, kidney damage in COVID-19 seems to be an AKI with non-selective glomerular proteinuria as the result principally of severe ATN. The lack of direct evidence of the presence of SARS-CoV-2 in the blood, urine and kidney tissue (according to RT-PCR, EM and IHC) leads us to conclude that Acute kidney injury (AKI) is probably induced indirectly by the systemic inflammatory response to the virus.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

We declare that the results presented in this article have not been published previously in whole or part, except in abstract format.

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