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Elevated Soluble Urokinase Plasminogen Activator Receptor (suPAR) is associated with renal dysfunction in a *Chlorocebus atheiops* COVID-19 model: *A Case Report*

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Abstract

Increases of soluble urokinase Plasminogen Activator Receptor (suPAR) were measured in both urine and plasma of a *Chlorocebus aethiops* (African Green Monkey; AGM) mucosal infected with SARS-CoV-2. The data indicate that elevated suPAR may be associated with renal dysfunction and pathology in the context of COVID-19.

Keywords

SARS-CoV-2; nonhuman primate; African Green Monkey; renal injury; acute kidney injury

1 | CLINICAL OBSERVATIONS

An approximately 7-yo male AGM (*JK41*) was mucosal inoculated with SARS-CoV-2 (WA/2020) via concomitant intratracheal/intranasal inoculation. SARS-CoV-2 was detected from

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

ETHICS APPROVAL

The Tulane University Institutional Animal Care and Use Committee approved all procedures used during this study. The Tulane National Primate Research Center (TNPRC) is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC No. 000594). The U.S. National Institutes of Health (NIH) Office of Laboratory Animal Welfare number for TNPRC is A3071-01. Tulane University Institutional Biosafety Committee approved all procedures for work in, and removal of samples from, Biosafety Level 3 laboratories.

mucosal swabs by $TCID_{50}$ (50% Tissue Culture Infection Dose) and sub-genomic RNA using PCR. Viral loading in mucosa and lung increased and peaked at 7-days post infection and resolved at 14-days post inoculation (DPI) and persisted throughout the course of the study in pharyngeal and nasal swabs as well as bronchial brush samples. The highest levels of viral RNA were detected in the pharynx and nasal cavity at 1E+7-1E+11 and 1E+8-1E+9 sgRNA per swab, respectively. The animal (JK41) was euthanized at 28-days post exposure. Clinical findings during the first 7-days post infection included mild transient changes in saturation pressure of oxygen and appetite were also recorded. Urine was negative for albumin, glucose, or acetone. No red blood cells and no cast elements were detected in urine during this time. For protein/creatinine ratio, urine suPAR and plasma suPAR values shown in Figure 1, an average of two measurements is reported.

2 | KIDNEY FUNCTION ANALYSIS

Urine and blood samples were taken at days 0 and 7. Blood urea nitrogen (BUN) and creatinine were measured as indicators of renal function. To further estimate renal dysfunction, we also measured urea/plasma creatinine ratio (Figure 1A-B). Both values were elevated at 7 DPI. Furthermore, protein/creatinine ratio was elevated in urine after 7 DPI (Figure 1C). No other alterations in urine were found. A slight decrease in hemoglobin and hematocrit levels were also found after 7 DPI (Table 1).

3 | SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (suPAR) IN PLASMA AND URINE INCREASED AFTER 7 DPI

The suPAR, results from the cleavage and membrane bound uPAR in inflammatory or immune mediated processes (Thuno et al., 2009). Indeed, changes in suPAR levels have been associated with the activation level of the immune system and can be used as a biomarker of immune activation in biological fluids, which is particularly important in kidney disease (Enocsson et al., 2020; Thuno et al., 2009). We found increased urine (Figure 1D) and plasma levels (Figure 1E) of suPAR after 7 DPI with SARS-CoV-2. These findings agree with previous reports in COVID-19 hospitalized patients showing increased suPAR in plasma even after increases in plasma creatinine, as indicator of renal dysfunction (Azam et al., 2020; Oulhaj et al., 2021)

4 | RENAL HISTOPATHOLOGICAL FINDINGS

During clinical evolution of the case, findings were compatible with decreased renal function. These features were associated with renal histopathological abnormalities as described in Figure 1F-H.

5 | DISCUSSION

The nonhuman primate and specifically the AGM species has been developed as an experimentally inducible mild to moderate animal model of COVID-19 (Munoz-Fontela et al., 2022). This species is useful in defining pathogenesis based investigations (Blair et al., 2021; Edwards et al., 2021; Rowe et al., 2022; Witt et al., 2021; Woolsey et al., 2020) and

associated re-infection susceptibility studies (Woolsey et al., 2021). Accordingly, changes in renal function and related pathologic consequences in an AGM COVID-19 model may mimic the human condition and disease outcomes. Acute kidney injury (AKI) commonly occurs in patients with COVID-19 and is often associated with respiratory complications (Ardura et al., 2022). The pathogenesis of AKI in the setting of COVID-19 infection is not well understood and biomarkers for its prediction are necessary to afford better clinical decisions. Increased suPAR levels have been identified as an immunologic risk factor for AKI and the existence of an association between suPAR and decreased renal function has been suggested (Azam et al., 2020). Indeed, increased plasma suPAR in hospitalized COVID-19 patients precedes increases in serum creatinine (Azam et al., 2020; Oulhaj et al., 2021) and it may predict even other renal complications such as renal damage associated to diabetes mellitus type 2 (Guthoff et al., 2017). Nonhuman primates are the closest animal models to humans to study physiology and pathophysiology playing an essential role in the current biomedical research related to virus infections (Heald et al., 2015) (Fahlberg et al., 2020). In this report, we describe that suPAR levels increase after 7 DPI with SARS-CoV-2 and this is also associated with increases in BUN, BUN/creatinine ratio and protein/creatinine ratio in urine, all of them indicators of reduced renal function. Increases in BUN are not only associated with kidney function. Pre-renal causes of renal dysfunction may be associated to intravascular volume alterations that may recapitulate acute renal failure and increased BUN levels (Molotoris et al., 2022). In fact, an increase in BUN can be expected in patients with pneumonia including those with COVID-19 as part of the CURB65 scoring (Wang et al., 2021). The BUN, albumin, and BUN)/albumin ratio (BAR) have been found to be reliable predictors of in-hospital mortality in COVID-19 patients, however BAR is a more reliable predictor than the BUN and albumin levels (Küçükceran et al., 2021).

In the case reported, renal dysfunction was associated with slight reduction in hematocrit and hemoglobin levels and without hematuria. Renal histology after 28 DPI of SARS-CoV-2 demonstrates changes consistent with glomerular congestion, focal mild glomerular capillary tuft collapse/shrinkage, diffuse mild tubular simplification and epithelial injury, such as flattening of the proximal tubular epithelium with loss of brush border as well as focal peritubular capillary and small arterial and venous congestion in AGM. Although the pathologic correlate of the sub-nephrotic proteinuria is not demonstrated, it is possible that the endothelial and /or epithelial injury could cause altered glomerular permeability in which the glomerular and microvascular congestion may a role in this. Furthermore, despite that the time interval was only 7-days, attempting biopsy samples on the glomeruli may yield additional information and evidence of glomerular cellular injury causing any degree of proteinuria (Zee et al., 2022). These changes further support similarities of these findings with kidney histopathology reported in humans with COVID-19 who underwent a renal biopsy (Akalin et al., 2020; Chan et al., 2020; Diao et al., 2021; Su et al., 2020) and highlight the relevance of non-human primate species as models of COVID-19-associated renal injury.

Although an explanation of the relationship between increases in suPAR and a decrease in kidney function with a single case report is a limitation of this study, we found clear evidence that several markers of renal dysfunction were affected at 7 DPI including BUN, BUN/plasma creatinine ratio, and urine protein/Creatinine ratio. However, suPAR is also

increased in inflammatory diseases such as pancreatitis (Küçükceran et al., 2018) thus it has been suggested as a biomarker of systemic inflammation (Rasmussen et al., 2021)which could be considered in the design of future studies.

Taken together, these findings suggest that the AGM COVID-19 model is useful to evaluate the development of kidney injury associated with COVID-19 and its progression. Equally important, the increases in urine and plasma suPAR levels during the SARS-CoV-2 viral infection suggest a severe disease outcome as observed in clinical hospitalized patients.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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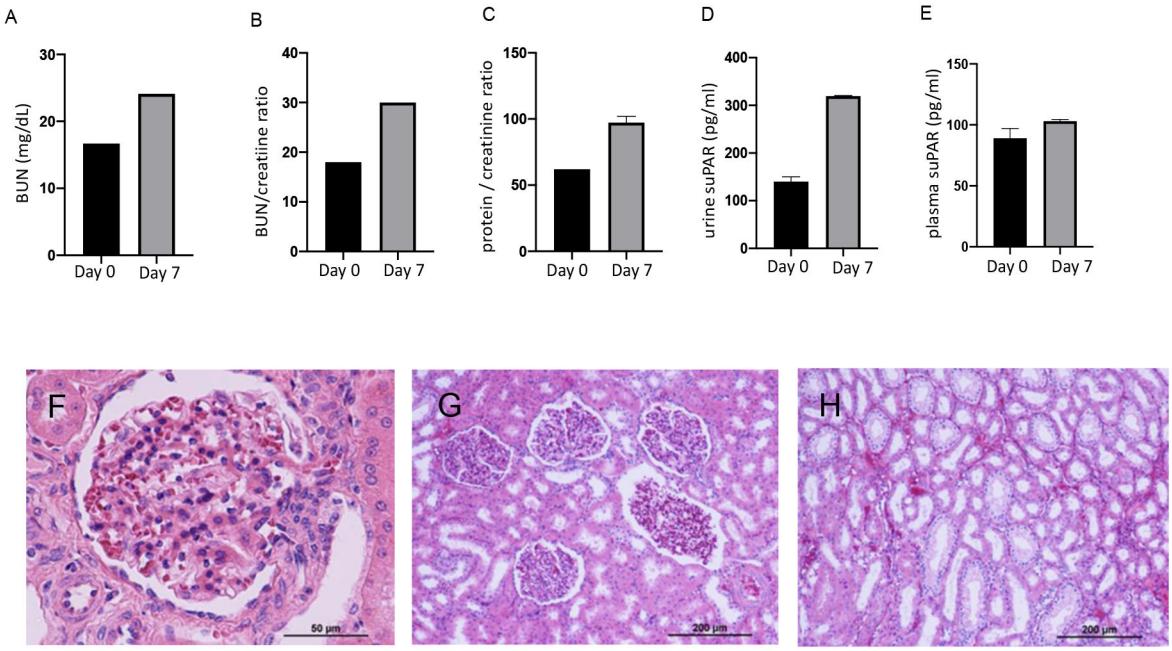

FIGURE 1.

Upper panels display analyses of clinical indicators of renal function and suPAR levels in plasma and urine samples at day 0 and after 7 days post infection: (A) blood urea nitrogen (BUN); (B) BUN / plasma creatinine ratio; (C) urine protein /Creatinine ratio; (D) urine suPAR (E) suPAR plasma levels. For protein/creatinine ratio, urine suPAR and plasma suPAR values, an average of two measurements is reported. **Lower panels display renal histopathological findings using hematoxylin and eosin staining of kidney sections (4 µm) from an AGM after 28 days of SARS-CoV-2 intranasal/intratracheal inoculation:** **(**F-H) show glomerular congestion (F), mild glomerular capillary tuft collapse/ shrinkage (F-G), diffuse mild tubular simplification/injury showing flattening of the proximal tubular epithelium with loss of brush border, focal peritubular capillary, small arterial and venous congestion (G-H). Microphotographs in panel F were captured using a 40x objective and G-H using a 10x objective. Scale is provided on each microphotograph.

TABLE 1

Blood parameters of AGM (JK41) at 0- and 7-days post inoculation (DPI).

	Day 0	Day 7
Hb (g/dL)	12.1	10.8
Hct (%)	36.1	32.5
Na^+ (mEq/L)	147.2	147.3
K^+ (mEq/L)	3.3	3.5
Cl^- (mEq/L)	106.3	107.4