
**Article Type:** Original Investigation

**Single-cell RNA sequencing of urinary cells reveals distinct cellular diversity in COVID-19-associated AKI**

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Matthew Cheung, Elise Erman, Shanrun Liu, Nathaniel Erdmann, Gelare Ghajar-Rahimi, Kyle Moore, Jeffrey Edberg, James George, and Anupam Agarwal

**Key Points:**
* Kidney alterations in AKI are challenging to study directly due to the need for kidney biopsy or post-mortem analysis to obtain cells.
* Urine scRNAseq can be used to noninvasively characterize cellular diversity and identify altered pathways in the setting of COVID-19 AKI.
* This study provides preliminary evidence that SARS-CoV-2 is capable of directly infecting urothelial cells.

**Abstract:**
Background: Acute kidney injury (AKI) is a common sequela of infection with SARS-CoV-2 and contributes to the severity and mortality from COVID-19. Here, we tested the hypothesis that kidney alterations induced by COVID-19-associated AKI could be detected in cells collected from urine. Methods: We performed single-cell RNA sequencing (scRNAseq) on cells recovered from the urine of eight hospitalized COVID-19 patients with (n=5) or without AKI (n=3) as well as four non-COVID-19 AKI patients (n=4) to assess differences in cellular composition and gene expression during AKI. Results: Analysis of 30,076 cells revealed a diverse array of cell types, most of which were kidney, urothelial, and immune cells. Pathway analysis of tubular cells from patients with AKI showed enrichment of transcripts associated with damage-related pathways compared to those without AKI. ACE2 and TMPRSS2 expression were highest in urothelial cells amongst cell types recovered. Notably, in one patient we detected SARS-CoV-2 viral RNA in urothelial cells. These same cells were enriched for transcripts associated with anti-viral and anti-inflammatory pathways. Conclusions: We successfully performed scRNAseq on urinary sediment from hospitalized patients with COVID-19 to noninvasively study cellular alterations associated with AKI and established a dataset that includes both injured and uninjured kidney cells. Additionally, we provide preliminary evidence of direct infection of urinary bladder cells by SARS-CoV-2. The urinary sediment contains a wealth of information and is a useful resource for studying the pathophysiology and cellular alterations that occur in kidney diseases.

**Disclosures:** A. Agarwal reports the following: Consultancy Agreements: Dynamed - my role is to review content related to AKI for Dynamed and review updated materials prepared by the Dynamed editorial team for AKI topics.; Akebia Therapeutics - I have been invited to serve on an Expert Panel to review new therapeutics based on the HIF pathway for AKI. I serve as a consultant for Reata Pharmaceuticals.; Ownership Interest: Goldlocks Therapeutics, Inc.; Research Funding: Genzyme/Sanofi Fabry Fellowship Award; Honoraria: Univ Southern California, Vanderbilt, Emory, Akebia therapeutics; Scientific Advisor or Membership: Editorial Board of AJP Renal, Kidney Int and Lab Investigation.; I have been invited to serve on the Advisory board of Goldlocks Therapeutics, a NY based company investigating delivery of drugs in the kidney using nanotechnology for acute and chronic kidney disease. I served on the External evaluation panel for the Kidney Precision Medicine Program (KPMP). I have been invited to serve on the Advisory Board of Angion and Alpha Young, LLC. The remaining authors have nothing to disclose.

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**Author Contributions:** Matthew Cheung: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing - original draft; Writing - review and editing Elise Erman: Data curation; Formal analysis; Writing - original draft; Writing - review and editing Shanrun Liu: Conceptualization; Methodology; Writing - review and editing Nathaniel Erdmann: Conceptualization; Data curation; Methodology; Writing - review and editing Gelare Ghajar-Rahimi: Data curation; Formal analysis; Investigation; Writing - review and editing Kyle Moore: Data curation; Formal analysis; Investigation; Jeffery Edberg: Conceptualization; Supervision; Writing - review and editing James George: Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Supervision; Validation; Writing - original draft; Writing - review and editing Anupam Agarwal: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources;
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Key Points

- Kidney alterations in AKI are challenging to study directly due to the need for kidney biopsy or post-mortem analysis to obtain cells.
- Urine scRNAseq can be used to noninvasively characterize cellular diversity and identify altered pathways in the setting of COVID-19 AKI.
- This study provides preliminary evidence that SARS-CoV-2 is capable of directly infecting urothelial cells.
Abstract

Background: Acute kidney injury (AKI) is a common sequela of infection with SARS-CoV-2 and contributes to the severity and mortality from COVID-19. Here, we tested the hypothesis that kidney alterations induced by COVID-19-associated AKI could be detected in cells collected from urine.

Methods: We performed single-cell RNA sequencing (scRNAseq) on cells recovered from the urine of eight hospitalized COVID-19 patients with (n=5) or without AKI (n=3) as well as four non-COVID-19 AKI patients (n=4) to assess differences in cellular composition and gene expression during AKI.

Results: Analysis of 30,076 cells revealed a diverse array of cell types, most of which were kidney, urothelial, and immune cells. Pathway analysis of tubular cells from patients with AKI showed enrichment of transcripts associated with damage-related pathways compared to those without AKI. ACE2 and TMPRSS2 expression were highest in urothelial cells amongst cell types recovered. Notably, in one patient we detected SARS-CoV-2 viral RNA in urothelial cells. These same cells were enriched for transcripts associated with anti-viral and anti-inflammatory pathways.

Conclusions: We successfully performed scRNAseq on urinary sediment from hospitalized patients with COVID-19 to noninvasively study cellular alterations associated with AKI and established a dataset that includes both injured and uninjured kidney cells. Additionally, we provide preliminary evidence of direct infection of urinary bladder cells by SARS-CoV-2. The urinary sediment contains a wealth of information and is a useful resource for studying the pathophysiology and cellular alterations that occur in kidney diseases.
Introduction

SARS-CoV-2 infection triggers pathology across multiple systems, including the kidney, and acute kidney injury (AKI) is associated with significant morbidity and mortality in Coronavirus Disease 2019 (COVID-19) (1–5). Multiple studies have demonstrated high rates of AKI amongst hospitalized patients with COVID-19 (6), with some reporting up to 50% of infected individuals developing AKI (7–9). The primary SARS-CoV-2 receptor ACE2 is expressed on epithelial cells throughout the urinary system, including proximal tubule cells and urothelial cells (10–13), although it is unclear if AKI in patients with COVID-19 is due to direct viral infection of the proximal tubules or is a result of the systemic response to SARS-CoV-2 (14–22). Similarly, it is unclear if SARS-CoV-2 can cause viral cystitis via direct infection of urothelial cells, although this possibility has been proposed (23–26). Despite the high prevalence of COVID-19-associated AKI, the underlying cellular alterations that occur in the setting of AKI remain poorly understood.

COVID-19-associated AKI has remained largely understudied as access to kidney tissue requires biopsy or post-mortem analysis (27). Recent studies have detected a diverse array of kidney, bladder, and immune cells in the urine. (28–30). Thus, the urine may offer valuable insight to noninvasively study kidney changes during COVID-19-associated AKI. Here, we performed single-cell RNA sequencing (scRNAseq) to characterize the cellular diversity in the urine of hospitalized COVID-19 patients with and without AKI. We tested the hypothesis that kidney alterations in COVID-19-associated AKI could be detected in cells collected from urine. We also collected samples from patients without COVID-19 and with AKI (non-COVID-19 AKI). We found several inflammatory immune cell populations and differentially activated pathways in COVID-19-associated AKI as well as preliminary evidence for direct infection of urothelial cells by SARS-CoV-2.
**Methods**

*Participants and Variables*

Adults aged 18 and older were screened during admission or transfer to the University of Alabama at Birmingham (UAB) hospital between March and May 2021. Cases of AKI were identified using the Kidney Disease Improving Global Outcomes (KDIGO) definition as a rise in serum creatinine (sCr) >0.3mg/dL within 48 hours or greater than 1.5x baseline creatinine. Controls with no change in creatinine were selected based on age and gender matching where possible and processed with each respective AKI sample. Baseline sCr was determined using the most recent sCr value 7-365 days prior to hospitalization. Additional clinical data regarding demographics, medical history, clinical characteristics, and laboratory values were extracted from patient charts through the UAB Center for Clinical and Translational Sciences (CCTS) i2b2 team. Eight hospitalized patients with COVID-19, 5 with AKI and 3 without AKI, were included. This study and specimen collections were approved by the UAB Institutional Review Board (IRB). The UAB Acute Nephrology Consult Team also collected samples from non-COVID-19 AKI patients (n=4) by to compare cellular changes with COVID-19-associated AKI. These were collected under a different IRB protocol that allows for collection of remnant urine samples and are thus anonymous.

*Specimen Collection and Processing*

All steps were performed on ice. Urine was collected as either a voided specimen or from a urinary catheter. Samples were immediately transferred to a biosafety level (BSL) 2+ laboratory for processing. Urine samples were transferred to a 50mL conical tube and centrifuged at 1000x g for 10 minutes at 4°C. Cell pellets were washed with ice-cold phosphate buffered saline (PBS), filtered through a 40µm filter, and centrifuged again. Live cells were purified using the MACS Debris Removal Kit (Miltenyi Biotec) followed by the EasySep Annexin V Dead Cell Removal Kit (StemCell). Briefly, cells were resuspended in ice-cold PBS and mixed with debris
removal solution. Cold PBS was overlaid on the mixture and centrifuged at 300x g for 10 minutes at 4°C. The top two phases were aspirated and then remaining cells were washed with PBS. Cells were resuspended and mixed with Dead Cell Removal Cocktail, Biotin Selection Cocktail, then RapidSpheres before separation in an EasySep magnet. Cells were washed and resuspended in 52µL of PBS (no calcium or magnesium) + 0.04% BSA (Fisher Scientific) for scRNAseq processing.

**Single Cell RNA Sequencing**

Purified cells were transferred to the UAB Flow Cytometry and Single Cell Core and immediately processed using the Chromium 3’ Single Cell RNA sequencing kit (10X Genomics) according to the manufacturer’s instructions. The cell suspension was counted and combined with 10X Chromium reagent mixture and loaded into a microfluidic single-cell partitioning device in which lysis and reverse transcription occur in microdroplets. The resulting cDNA was amplified by polymerase chain reaction and subsequently processed to yield bar-coded sequencing libraries. Paired-end sequencing was carried out on Illumina NovaSeq6000 or NextSeq500 sequencing platforms (Illumina). Reads were processed using the 10x Genomics Cell Ranger Single-Cell Software Suite version 6.0 on the UAB Cheaha High Performance Computing Cluster. BCL files were converted to FASTQ files using the CellRanger mkfastq function. CellRanger mkfastq was used to align FASTQ files to a custom genome consisting of the hg38 human genome (GRCh38.p13) with the SARS-CoV-2 genome (NC_045512.2) inserted as an exon (31). The genes table, barcodes table, and transcriptional expression matrices were created for the analysis indicated below.

**Data Analysis**

Analyses were carried out using packages created for the R statistical analysis environment (version 4.06). Data were primarily analyzed in Seurat version 3.2.3 (32,33) and its associated
dependencies. Data from each individual patient were imported using the Read10X function and then structured into a Seurat object using CreateSeuratObject. For quality control, cells with unique feature counts over 2,500 or under 200 and cells with mitochondrial proportions greater than 15% were filtered out. Data were normalized using LogNormalize and scaled to prepare for linear dimensional reduction. Objects from individual patients were labeled with unique group IDs and then merged into a single object using the Seurat merge function. Patient samples were integrated with the RunHarmony function using the Harmony R package (34). Principal component analysis was performed and then cells were clustered based on differential gene expression as determined by the Seurat FindAllMarkers function set to a resolution of 1. Dimensional reduction was performed using uniform manifold approximation and projection (UMAP). Cell types were identified by comparing the differentially expressed transcripts for each cluster to known transcripts associated with specific cell types (29,35,36). The Escape R package was used to run Gene Set Enrichment Analysis (GSEA) (37). WebGestaltR was used for gene ontology analysis to identify pathways using the Biologic Process and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (38).

Data Availability

The scRNAseq data generated in this paper are available in GEO under accession number GSE180595.

Results and Discussion

We performed scRNAseq on urine sediment from eight hospitalized patients with COVID-19, five with AKI and three without AKI. Four additional urine samples from hospitalized patients without COVID-19 and with AKI were also collected to compare potential differences in AKI in the context of COVID-19. The average sCr in patients with COVID-19-associated AKI was 2.4 mg/dL as compared to 0.83 mg/dL in control patients at the time of collection. Notably, two of
the patients with COVID-19-associated AKI had prior diagnoses of chronic kidney disease (CKD), which has been shown to worsen outcomes from COVID-19 (39,40). Comorbidities and other variables differed slightly between the AKI and no AKI control populations (Table 1).

Consistent with prior reports (29), cell numbers were highly heterogeneous between patients (Supplemental Figure 1A and B). In total, 65,234 cells were sequenced, and 30,076 cells were retained through quality control. Integrated UMAPs of all 12 patient samples revealed a diverse array of kidney, urothelial, and immune cells (Figure 1A, E, F). We were able to detect most kidney cells, including podocytes and VCAM1+ kidney cells, demonstrating the ability to noninvasively study the kidney through analysis of urine. There is clear heterogeneity in the urothelial cell population, which falls outside the scope of this study, but warrants further investigation. The cell types captured varied between AKI and non-AKI groups (Figure 1B, C, and D) as well as between each patient (Supplemental Figure 1C). As expected, a larger number of immune cells were detected in the COVID-19-associated AKI samples.

GSEA analysis revealed upregulation of apoptotic genes, interferon response elements, and various other signaling pathways in both COVID-19-associated and non-COVID-19 AKI and non- AKI control samples (Supplemental Figure 2 and Figure 2C). Due to the high energy requirements and mitochondrial content, the proximal tubule is one of the structures most sensitive to insult during AKI (41) and its severity of injury is related to overall outcomes (42). Pathway analysis of proximal tubule cells from patients with AKI revealed enrichment of pathways related to apoptosis, DNA repair, and cellular responses to reactive oxygen species (ROS) and stress in contrast to patients without AKI (Figure 2A and B). Similar pathway upregulation was detected in other kidney tubular cells. Pathways related to damage were upregulated in the setting of AKI, but enrichment of ROS, DNA damage, and apoptotic pathways in the proximal tubules, loop of Henle, and collecting duct were more apparent in
COVID-19-associated AKI in comparison to the non-COVID-19 AKI samples. We suspect that there are many overlapping pathways in AKI due to the combination of injury mechanisms including ischemia, inflammation, or drug toxicity. The additional enrichment of damage-related pathways in COVID-19-associated AKI may be due to the other inflammatory processes occurring in the COVID-19 disease process. This may explain the metabolic differences seen in urine of patients with COVID-19-associated AKI (43).

The viral entry receptor, ACE2, is abundantly present in a variety of tissues including the kidneys. ACE2 and TMPRSS2 transcripts were detected on various epithelial cells from the kidney and were particularly abundant in urothelial cells (Figure 3A). Since SARS-CoV-2 is an RNA virus with poly-A tail (44), we created a customized genome for alignment where the SARS-CoV-2 genome was added as an exon to the hg38 human genome. Interestingly, while we did not detect any SARS-CoV-2 viral transcripts in the kidney cells in patients with AKI, we did detect trace transcripts in the urothelial cells of Patient 7 (who had COVID-19 without AKI), suggesting direct infection of these bladder cells (Figure 3A). Individual analysis of Patient 7 revealed few kidney-derived cells. Most of the cells isolated were immune and urothelial cells. Viral RNA was found exclusively in urothelial cells with high ACE2 expression (Figure 3B, Supplemental Figure 3). Urinalysis of this patient showed leukocyturia without evidence of a bacterial infection (negative leukocyte esterase and nitrite) (Figure 3C). Pathway analysis of the urothelial cells revealed enrichment of pathways involved in type 1 interferon signaling, an acute inflammatory response, and leukocyte responses (Figure 3D and Supplemental Figure 2B) which is consistent with a viral infection of these cells.

Lack of detection of viral RNA in proximal tubule cells in the urine does not rule out direct infection by SARS-CoV-2. Other groups have reported evidence for and against viral tropism in the kidney (14–22). The detection of viral RNA in urothelial cells and the enrichment of
transcripts associated with antiviral pathways in these cells suggests direct infection and a possible case of viral cystitis. Viral cystitis due to SARS-CoV-2 has been noted, but evidence of direct infection has been lacking. The mechanism of entry would be from the basal side of the urothelial cells if from the blood or luminal side if from the urine. A previous study suggested that ACE2 is expressed both on the basal and luminal surface of the urinary bladder (23). The detection of viral cystitis using scRNAseq is consistent with the clinical characteristics from Patient 7 (Figure 3C). While the virus was only detected in a small number of cells, we suspect the majority of virally infected cells could have been filtered out during the urine processing and data analysis quality control steps in which we removed dead and dying cells.

A limitation of this study is the small number of patients sampled. Sample collections were restricted to new hospital admissions to control for other variables such as the various medications used as treatments for COVID-19. Hospitalized COVID-19 patients without AKI were used as controls because healthy controls do not secrete many cells in urine. Additional limitations include having to process urine before sequencing; however, this greatly increased the proportion of live cells obtained. Here, cells were readily detectable in the urine of infected patients without kidney injury. This is significant because most scRNAseq studies have focused on damaged kidneys leading to few data sets available to study uninjured kidney cells. AKI has many different causes and only a subset may be caused by direct kidney infection by SARS-CoV-2, making it difficult to draw broad conclusions about AKI in COVID-19. Additionally, it is possible that direct viral infection of kidney cells occurs later in the infection process than was captured in this study. Subsequent studies will investigate urinary cell changes over the course of illness with sequential samples taken before, during, and after AKI.

We successfully performed scRNAseq on urinary sediment from hospitalized patients with COVID-19. This allowed noninvasive studies of cellular changes occurring during AKI, a
frequent manifestation of COVID-19. The urinary cell composition and upregulated pathways in hospitalized COVID-19 patients drastically differ in those with AKI versus those without AKI. We also provide preliminary evidence of a potential case of viral cystitis through direct infection of urinary bladder cells. Analysis of urinary cells may provide a useful avenue to understanding the pathophysiology and cellular alterations that occur in kidney diseases.

**Disclosures**

A. Agarwal reports the following: Consultancy Agreements: Dynamed - my role is to review content related to AKI for Dynamed and review updated materials prepared by the Dynamed editorial team for AKI topics.; Akebia Therapeutics - I have been invited to serve on an Expert Panel to review new therapeutics based on the HIF pathway for AKI. I serve as a consultant for Reata Pharmaceuticals.; Ownership Interest: Goldilocks Therapeutics, Inc.; Research Funding: Genzyme/Sanofi Fabry Fellowship Award; Honoraria: Univ Southern California, Vanderbilt, Emory, Akebia therapeutics; Scientific Advisor or Membership: Editorial Board of AJP Renal, Kidney Int and Lab Investigation.; I have been invited to serve on the Advisory board of Goldilocks Therapeutics, a NY based company investigating delivery of drugs in the kidney using nanotechnology for acute and chronic kidney disease. I served on the External evaluation panel for the Kidney Precision Medicine Program (KPMP). I have been invited to serve on the Advisory Board of Angion and Alpha Young, LLC. The remaining authors have nothing to disclose.

**Funding**

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Author Contributions

Matthew Cheung: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing - original draft; Writing - review and editing. Elise Erman: Data curation; Formal analysis; Writing - original draft; Writing - review and editing. Shanrun Liu: Conceptualization; Methodology; Writing - review and editing. Nathaniel Erdmann: Conceptualization; Data curation; Methodology; Writing - review and editing. Gelare Ghajar-Rahimi: Data curation; Formal analysis; Investigation; Writing - review and editing. Kyle Moore: Data curation; Formal analysis; Investigation. Jeffrey Edberg: Conceptualization; Supervision; Writing - review and editing. James George: Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Supervision; Validation; Writing - original draft; Writing - review and editing. Anupam Agarwal: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing - original
draft; Writing - review and editing. All authors reviewed and approved the manuscript prior to submission.

**Supplemental Materials**

Supplemental Figure 1. Heterogeneity of cells captured from each patient sample.

Supplemental Figure 2. Gene Set Enrichment Analysis (GSEA) of hallmark pathways in both AKI and no AKI control clusters.

Supplemental Figure 3. Heatmap of differentially expressed genes from Patient 7 cells.

**References**


Table 1. Baseline characteristics and demographics of study participants. AKI was defined using KDIGO criteria of rise in serum creatinine of >0.3mg/dL or 1.5x baseline. Clinical data from nonCOVID-19 AKI patients (Patients 9-12) were not obtained as samples were collected anonymously.

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Figure 1. Single-cell RNA sequencing of urine from COVID-19 patients with and without AKI and non-COVID AKI. A) Integrated UMAP plot of 30,076 cells sequenced from patient urine sediment. B,C and D) UMAP plot subsetted by COVID-19 and AKI status showing the difference in collected cell populations. Cell type labels are the same. E) Heatmap of differentially expressed genes of integrated clusters F) Dot plot of known marker genes for identification of cells.
Abbreviations: CD; collecting duct, Epi; epithelial, KRM; kidney resident macrophage, LOH; loop of Henle, Lympho; lymphocytes, Mac; macrophage, Mes; mesenchymal, Neut; neutrophil Podo; podocyte, Prolif; proliferating, PT; proximal tubule, RBC; red blood cell, Ribo; ribosomal, SMC; smooth muscle cell, Uro; urothelial

Figure 2. Enrichment of damage-related pathways AKI. Gene set enrichment analysis of each cluster of cells from the integrated UMAP for pathways including A) hallmark reactive oxygen species pathways, B) hallmark apoptosis pathways, and C) hallmark DNA repair pathways in COVID-19 AKI (left), COVID-19 no AKI controls (middle), and non-COVID-19 AKI (right). Cluster identifications: 1-Urothelial, 2-Urothelial, 3-Urothelial, 4-Macrophage, 5-Major histocompatibility complex class II positive neutrophil, 6-Loop of Henle, 7-Neutrophil, 8-Collecting Duct, 9-Urothelial, 10-Umbrella, 11-Neutrophil, 12-Collecting Duct, 13-Kidney resident macrophage, 14-Neutrophil, 15-Urothelial, 16-Cycling, 17-Proximal tubule, 18-Proximal tubule, 19-Smooth muscle cell, 20-Intercalated cell, 21-Urothelial, 22-T cell, 23-Podocyte, 24-Red Blood Cell, 25-Macrophage, 26-B cell, 27-Macrophage, 28-Red blood cell

Figure 3. Detection of direct infection of urothelial cells by SARS-CoV-2. A) Violin plots of ACE2, TMPRSS2 and SARS-CoV-2 gene expression amongst sequenced cell types. Cell types with identified SARS-CoV-2 gene expression are identified in blue to identify overlap between SARS-CoV-2 receptor and gene expression. B) Individual analysis of Patient 7: UMAP plots, and ACE2 and SARS-CoV-2 expression identifying presence of SARS-CoV-2 gene expression within urothelial cells C) Urinalysis labs from urine samples taken from Patient 7 two days prior and twelve days after urine collection for scRNAseq. D) Gene enrichment analysis using WebGestaltR Biologic Process to identify upregulated pathways in the urothelial cells of Patient 7. Pathways involved in viral infection and immune response are shown in blue with a false discovery rate (FDR) of less than or equal to 0.05. Abbreviations: CD; collecting duct, Epi; epithelial, KRM; kidney resident macrophage, LOH; loop of Henle, Lympho; lymphocytes, Mac; macrophage, Mes; mesenchymal, Neut; neutrophil Podo; podocyte, Prolif; proliferating, PT; proximal tubule, RBC; red blood cell, Ribo; ribosomal, SMC; smooth muscle cell, Uro; urothelial
Figure 2

A. Hallmark Reactive Oxygen Species Pathways

B. Hallmark Apoptosis Pathways

C. Hallmark DNA Repair Pathways
Figure 3

A

ACE2

TMPRSS2

SARS-CoV-2

B

0,1,2-Uro

KRT13

CD45

ACE2

SARS-CoV-2

C

2 days prior 12 days after

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<tr>
<td>Sq Epi</td>
<td>Rare</td>
<td>Normal</td>
</tr>
<tr>
<td>SG</td>
<td>1.027</td>
<td>1.019</td>
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<tr>
<td>Protein</td>
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<td>2+</td>
</tr>
<tr>
<td>pH</td>
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<td>5</td>
</tr>
<tr>
<td>Nitrite</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Culture</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Leuk Est</td>
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<td>Neg</td>
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<tr>
<td>Glucose</td>
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<td>Neg</td>
</tr>
<tr>
<td>Ketone</td>
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<td>Neg</td>
</tr>
<tr>
<td>Color</td>
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<td>Yellow</td>
</tr>
<tr>
<td>Clarity</td>
<td>Clear</td>
<td>Slightly cloudy</td>
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<tr>
<td>Blood</td>
<td>Negative</td>
<td>1+</td>
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D

virus attachment to host cell
negative regulation of retinol metabolism process
keratinization
regulation of blood coagulation
extrinsic apoptotic signaling pathway via death domains
antimicrobial humoral response
regulation of cell killing
regulation of epidermis development
keratinocyte differentiation
retinol metabolic process
negative regulation of blood coagulation
cornification
monocyte chemotaxis
neutrophil chemotaxis
neutrophil activation involved in immune response
epidermal cell differentiation
regulation of endopeptidase activity
leukocyte chemotaxis

No AKI – Urothelial Cells

FDR ≤ 0.05
### Supplemental Materials Table of Contents

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Supplemental Figure 1. Heterogeneity of cells captured from each patient sample. A) Number of cells sequenced from each patient with COVID-19 AKI (red), without AKI (black), or with non-COVID-19 AKI (blue). B) Breakdown of various patient cell contributions to integrated UMAP. C) Breakdown of COVID-19 AKI (red) and no AKI control (blue), and non-COVID-19 AKI (green) contribution to integrated UMAP.
Supplemental Figure 2. Gene Set Enrichment Analysis (GSEA) of hallmark pathways in both AKI and no AKI control clusters. A) Up and downregulation of various hallmark pathways in COVID-19 AKI (orange), no AKI control (green), and non-COVID-19 AKI (blue) clusters. B) Up and downregulation of interferon alpha response pathways in each individual cell type in COVID-19 AKI (blue), no AKI control (turquoise), and non-COVID-19 AKI (red).
Supplemental Figure 3. Heatmap of differentially expressed genes from Patient 7 cell clusters.