**Outbreak of SARS-CoV-2 in hospitalized hemodialysis patients: an epidemiologic and genomic investigation**

**Supplemental Material**

Outbreak Investigation and Response

Cases were identified through daily surveillance of all positive COVID results among inpatients by infection prevention. The outbreak was suspected due to positive COVID-19 testing results among several patients on the inpatient renal specialty ward. A line list was generated to assess for common risk factors and exposures. None other than hemodialysis was identified.

The inpatient renal specialty ward consists of 24 single-patient rooms (Supplementary Figure 1) where patients are assigned for their overall stay. Patients are then temporarily transported to the hemodialysis unit, comprised of 16 open air bays, in another building for treatment.  Infection prevention policy is that curtains be drawn, and patients wear masks while undergoing hemodialysis.

Exposure was defined as per CDC guidance (<https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-risk-assesment-hcp.html>). Testing was offered to all patients who had overlapped with case patients and who were still present on the unit at time of outbreak investigation. In addition, testing was offered, but not mandated, to all exposed HCWs. As per CDC guidance at the time, HCW that were asymptomatic were allowed to continue working but everyone was required to wear masks.

Library preparation and sequencing

Residual nasopharyngeal (NP) swab samples were retrieved from 4°C storage in the clinical laboratory and stored at −80°C until sequencing. Samples underwent RNA extraction, library construction, Illumina sequencing, and reference-based SARS-CoV-2 genome assembly as previously described [1] [2]. Briefly, RNA was extracted from primary samples, and repeat SARS-CoV-2 testing was performed by triplex RT-PCR [1]. Samples underwent RNA mNGS as previously described [2]. Methods included DNase treatment, random primer cDNA synthesis, and Nextera XT tagmentation. A water sample was included as a negative control with each library construction batch, and stringent laboratory protocols were followed to minimize the risk of contamination. Libraries were made with unique dual indexes.

Phylogenetic Analysis

SARS-CoV-2 genomes from 10 non-outbreak patients in the same facility and 58 patients in nearby facilities between 12/12/2020 and 1/13/2021 were aligned with 391 publicly available sequences from individuals in the state of Georgia from the same period, and single nucleotide polymorphisms (SNPs) were identified using Geneious Prime (v2021.0.3). Maximum-likelihood phylogenetic trees were generated under a generalized time-reversible model with 1,000 bootstrap replicates using IQtree v2.0.3 [3] and visualized and annotated using Interactive Tree of Life (iTOL) v4 [4].

References:

1. Waggoner JJ, Stittleburg V, Pond R, et al. Triplex Real-Time RT-PCR for Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis **2020**; 26(7): 1633-5.

2. Babiker A, Bradley HL, Stittleburg VD, et al. Metagenomic sequencing to detect respiratory viruses in persons under investigation for COVID-19. J Clin Microbiol **2020**.

3. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution **2014**; 32(1): 268-74.

4. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Research **2019**; 47(W1): W256-W9.

**Supplementary Fig. 1.** Hemodialysis and inpatient renal ward unit floor plan and location of involved patients and healthcare workers.(A) Patients are temporarily transported to the hemodialysis unit, which comprises 16 open air bays. On December 26, 2020, the presumed index patient (case 1) received dialysis in bay 5. Of the 7 other positive patients, 5 (cases 2–5 and 7) were in the hemodialysis in close proximity to case 1. Notably, case 5 started hemodialysis shortly after case 1 had completed dialysis in bay 11, which had previously been used by case 2. (B) The renal specialty ward consists of 26 single-patient rooms and is where patients are assigned for their overall stay. Cases 1, 2, 3, 4, and 6 were bedded in the same inpatient renal ward prior to and following December 26, 2020. Horizontal bars indicate HCWs with positive SARS-CoV-2 tests who cared for several of the cases: cases 9 (yellow), 11 (green), and 12 (blue). Red asterisks indicate patients from whom SARS-COV-2 genomes were successfully sequenced and identical, and blue asterisks indicate samples from whom no SARS-CoV-2 sequence was available.

**Supplementary Fig. 2.** Epidemiological analysis of the 13 outbreak cases. Exposure and onset of symptoms for 8 patients with hospital-onset COVID-19 (cases 1–8) and 5 exposed healthcare workers (cases 9–13).

**Supplementary Fig. 3.** Viral genomic analysis outbreak related cases and samples from the same facility. Maximum-likelihood phylogenetic tree of SARS-CoV-2 genomes from 6 individuals in this outbreak investigation (red), as well as 10 patients in the same facility and 58 patients in nearby facilities (blue), and 391 publicly available SARS-CoV-2 genomes from Georgia between December 12, 2020, and January 13, 2021.

**Supplementary Table 1.** GISAID Accession Numbers for the Publicly Available Sequences Used in This Analysis