SARS-CoV-2 in Spent Dialysate from Chronic Peritoneal Dialysis Patients with COVID-19

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Abstract

Background To date, it is unclear whether SARS-CoV-2 is present in spent dialysate from patients with COVID-19 on peritoneal dialysis (PD). Our aim was to assess the presence or absence of SARS-CoV-2 in spent dialysate from patients on chronic PD who had a confirmed diagnosis of COVID-19.

Methods Spent PD dialysate samples from patients on PD who were positive for COVID-19 were collected between March and August 2020. The multiplexed, real-time RT-PCR assay contained primer/probe sets specific to different SARS-CoV-2 genomic regions and to bacteriophage MS2 as an internal process control for nucleic acid extraction. Demographic and clinical data were obtained from patients’ electronic health records.

Results A total of 26 spent PD dialysate samples were collected from 11 patients from ten dialysis centers. Spent PD dialysate samples were collected, on average, 25 ± 13 days (median, 20; range, 10–45) after the onset of symptoms. The temporal distance of PD effluent collection relative to the closest positive nasal-swab RT-PCR result was 15 ± 11 days (median, 14; range, 1–41). All 26 PD effluent samples tested negative at three SARS-CoV-2 genomic regions.

Conclusions Our findings indicate the absence of SARS-CoV-2 in spent PD dialysate collected at ≥ 10 days after the onset of COVID-19 symptoms. We cannot rule out the presence of SARS-CoV-2 in spent PD dialysate in the early stage of COVID-19.

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Introduction

Currently, individuals suspected of coronavirus disease 2019 (COVID-19) are tested for the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid, mainly in nasopharyngeal-swab, oropharyngeal-swab, nasopharyngeal-aspirate, and bronchoalveolar-lavage specimens. SARS-CoV-2 was also detected in the blood of patients positive for COVID-19 (1,2). Viremia of SARS-CoV-2 has been proposed as a predictor of a severe clinical course (3,4).

Patients with kidney disease who are treated with peritoneal dialysis (PD) are a high-risk, vulnerable population. Previously, several viruses have been detected in spent PD dialysate, including hepatitis C virus and HIV (5,6). Data on the presence or absence of SARS-CoV-2 in spent PD dialysate are scarce; to date, results from only five patients on chronic PD have been reported. SARS-CoV-2 was found to be absent in four patients (7,8) and present in one (9). Therefore, it is currently unclear whether SARS-CoV-2 is present in spent PD dialysate, causing uncertainty and concerns for patients and their caregivers. To address these concerns, we collected and tested serial PD dialysate samples from patients on PD with confirmed COVID-19 for the presence of SARS-CoV-2.

Materials and Methods

Patient Selection

Collection of spent peritoneal dialysate from patients on PD who were positive for SARS-CoV-2 was done as a part of a quality-improvement project. The quality-improvement project was approved by the respective clinic governing bodies after legal and compliance review. Patients on PD who were positive for SARS-CoV-2 were contacted by US Fresenius Medical Care North America (FMCNA) healthcare professionals, who assisted with collecting the spent dialysate. Spent peritoneal dialysate was collected from patients on PD with a confirmed, positive, nasopharyngeal-swab test for SARS-CoV-2 from March until August 2020. Up to five spent PD dialysate samples per patient were collected. Patients received collection kits with

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instructions to leave gel ice packs in the freezer until the samples were collected and ready for shipment. Demographic and clinical data were retrospectively obtained from the patients' electronic health records. Verbal consent was obtained from all participants. The dialysate shipments to Renal Research Institute complied with all of the respective federal and state regulations. All samples were shipped overnight with ice packs.

RNA Extraction and Real-Time RT-PCR

RNA was extracted from 400 μl of spent PD dialysate and eluted in 50 μl of elution buffer, using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (A42352; Thermo Fisher) on the KingFisher Flex Purification System (5400630; Thermo Fisher). MS2 Phage Control was added to each sample, before RNA extraction, as an indicator of successful RNA extraction. Additionally, RNA was extracted from 2 ml of spent PD dialysate using the Qiagen QIAamp Viral RNA Mini Kit (52906; Qiagen), according to the instructions in the manual. MS2 phage control was added before RNA extraction. Final RNA was eluted in 30 μl of elution buffer. The PCR reaction mix was prepared using the TaqPath RT-PCR COVID-19 Combo Kit (A47814; Thermo Fisher). This kit contains probes that anneal to three specific SARS-CoV-2 target sequences: N protein, S protein, and ORF1ab. Diluted TaqPath COVID-19 Control (50 copies per PCR reaction) was used as a positive control. We used 5 μl of purified RNA from each sample for RT-PCR reaction on the QuantStudio 6 Pro Real-Time PCR System (A43159; Thermo Fisher). For each run, we used the same settings in the RT-PCR program as those provided in the instructions for the TaqPath RT-PCR COVID-19 Combo Kit. We performed 40 cycles of PCR amplification to achieve maximum detectability.

Statistical Analyses

Continuous variables are presented as mean±SD (range). Categoric variables are expressed as counts.

Results

We collected a total of 26 spent PD dialysate samples from 11 patients on PD from ten US FMCNA dialysis centers. Demographic and clinical data are shown in Table 1. Patients contributed an average of 2.4±1.4 samples (range, 1–5). Spent PD dialysate samples were collected, on average, 25±13 days (range, 10–45) after the onset of symptoms. The temporal distance of PD effluent (PDE) collection relative to the closest positive nasopharyngeal-swab RT-PCR test was 15±11 days (median, 14; range, 1–41); the time difference between PDE collection and the onset of symptoms for all 26 samples was 36±17 days (range, 10–75) (Figure 1). All PDE samples tested negative for SARS-CoV-2 RNA after 40 cycles of RT-PCR amplification. Of note, in patients 1, 3, and 11, RT-PCR tests of PDE were negative, although these patients were still considered positive for SARS-CoV-2, as confirmed by repeated nasopharyngeal-swab RT-PCR tests. The limit of detection is ten genomic copy equivalents (GCE) per PCR reaction, or 250 GCE/ml in PDE before RNA extraction. To enhance the detectability of SARS-CoV-2 RNA in PDE, we purified and concentrated RNA 66 times using the Qiagen RNA purification kit. The SARS-CoV-2 RNA testing result remained negative after 40 cycles of RT-PCR amplification. The calculated limit of detection was 30 GCE/ml in PDE in this case.

Discussion

Our results, determined on the basis of the largest cohort of patients with COVID-19 on chronic PD to date, indicate that SARS-CoV-2 was not present in the spent PD dialysate collected in our population ≥10 days after the onset of symptoms. In our patients, COVID-19 was diagnosed by RT-PCR of nasopharyngeal-swab samples. The findings corroborate previous results in four patients on chronic PD reported by other groups (7,8). To the best of our knowledge, the report by Vischini et al. (9) is the only one finding a positive SARS-CoV-2 test in spent PD dialysate from a patient on chronic PD. The patient was diagnosed with COVID-19 and hospitalized for 40 days. Her PDE tested positive for SARS-CoV-2 multiple times, even at the time of discharge. Because viral culture was absent, it is unknown if this finding indicates viable SARS-CoV-2 or mere RNA remnants. Alternatively, the patient may have had an unusually high viral load throughout the illness (10). However, that is not typical. A virologic assessment study, published in April 2020, demonstrated that pharyngeal virus shedding was very high during the first week of symptoms, peaking at 7.11×10⁸ RNA copies per throat swab on day 4 (10). No live virus could be isolated from pharyngeal samples taken 8 days after the onset of symptoms, in spite of ongoing high viral load (10).

It is important to consider the timing of PD dialysate collection relative to the onset of symptoms. In our patients, the earliest PD dialysate collection was 10 days after the

<table>
<thead>
<tr>
<th>Variables</th>
<th>Results</th>
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<tbody>
<tr>
<td>Patients (n)</td>
<td>11</td>
</tr>
<tr>
<td>Age, yr</td>
<td>54±11 (34–69)</td>
</tr>
<tr>
<td>Females (n)</td>
<td>8</td>
</tr>
<tr>
<td>Time on PD, mo</td>
<td>16.1±15.9 (1–58)</td>
</tr>
<tr>
<td>PD modality (CCPD/CAPD), n</td>
<td>10/1</td>
</tr>
<tr>
<td>Hospitalized due to COVID-19, n</td>
<td>3</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean±SD (range). Categoric variables are expressed as counts. PD, peritoneal dialysis; CCPD, continuous cycling peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis; COVID-19, coronavirus disease 2019.
onset of symptoms (patient 4). Therefore, we cannot rule out the presence of SARS-CoV-2 in the early stage of the disease. Candellier et al. (7,11) collected spent PD dialysate from three patients at 0, 3, 4, and 7 days after hospital admission; none of the samples tested positive for SARS-CoV-2, despite high initial viral loads (cycle threshold <30). Sadioglu et al. (8) reported on two PDE samples collected from a patient on days 3 and 4 after the onset of symptoms, and both tested negative for SARS-CoV-2. Similar to the above findings in patients on chronic PD, El Shamy et al. (12) reported no SARS-CoV-2 present in the spent dialysate of ten patients, hospitalized with COVID-19, who had acute kidney injuries that were treated with acute PD.

Coccolini et al. (13) reported on a surgical patient with SARS-CoV-2 and intact kidney function who suffered from small bowel volvulus and underwent emergency surgery. Barberis et al. (14) also reported on a patient severely ill with COVID-19 who underwent surgery. In both cases, RT-PCR of intraoperatively collected peritoneal fluid tested positive for SARS-CoV-2. Considering the circumstances, contamination from blood or other body fluid could be one source of the positive signal.

Only a small fraction of patients that tested positive for SARS-CoV-2 are viremic, and those are typically patients with severe COVID-19 symptoms (1–4). With a size of 60–140 nm, SARS-CoV-2 could theoretically enter the peritoneal cavity via translocation from the blood (11,15). Therefore, it could be argued that patients on PD with severe COVID-19 are more likely to be viremic and, thus, have potentially contagious spent dialysate. Viremic blood samples are not necessarily contagious, however. For example, a recent medRxiv preprint reported a study of 20 blood samples containing SARS-CoV-2 RNA; in viral cultures, no viral replication was detected, suggesting these blood samples were not infectious (M. Andersson et al., unpublished observations). To definitively answer the question of contagiousness of spent peritoneal dialysate, longitudinal studies—which include repeated RT-PCR testing and viral cultures of spent peritoneal dialysate from symptom onset to recovery—are warranted.

Disclosures
J.E. Chao, G.F. Dias, N. Grobe, M. Hakim, M. Han, O. Thwin, Z. Haq, P. Kotanko, A. Patel, P. Preciado, J. Raimann, X. Tao, L. Tisdale, X. Wang, and X. Ye are employees of the Renal Research Institute, a wholly owned subsidiary of FMCNA. D. Chatoth and R. Lasky are employees of FMCNA. D. Chatoth and P. Kotanko hold stock in Fresenius Medical Care. All remaining authors have nothing to disclose.

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Author Contributions
J.E. Chao, G.F. Dias, N. Grobe, M. Hakim, Z. Haq, A. Patel, P. Preciado, and X. Wang were responsible for investigation; D. Chatoth, N. Grobe, P. Kotanko, J. Raimann, and X. Wang reviewed and edited the manuscript; D. Chatoth, P. Kotanko, A. Patel, O. Thwin, and L. Tisdale were responsible for resources; N. Grobe, P. Kotanko, and X. Tao provided supervision; N. Grobe, P. Kotanko,
X. Tao, and X. Wang conceptualized the study; N. Grobe, A. Patel, and X. Wang wrote the original draft; M. Han, A. Patel, P. Preciado, X. Tao, O. Thwin, and L. Tisdale were responsible for project administration; R. Lasky and J. Raimann were responsible for data curation; X. Wang was responsible for methodology; X. Wang and X. Ye were responsible for formal analysis; and all authors provided critical feedback and helped shape the research, analysis, and manuscript.

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