






RESEARCH ARTICLE

Dynamics of spreading of SARS-CoV-2 in a Belgian hemodialysis facility: The importance of the analysis of viral strains

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Abstract

In-center maintenance hemodialysis (HD) patients are at high risk of acquiring coronavirus disease 2019 (COVID-19) by cross-contamination inside the unit. The aim of this study was to assess retrospectively the dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission during the very first pandemic phase (March–July 2020) in a cohort of in-center maintenance HD patients and in nurses the same HD facility, using a phylogenetic approach. All SARS-CoV-2 quantitative reverse-transcription polymerase chain reaction positive patients and nurses from our HD unit-respectively 10 out of 98, and 8 out of 58- and two other positive patients dialyzed in our self-care unit were included. Whole-genome viral sequencing and phylogenetic analysis supported the cluster investigation. Five positive patients were usually dialyzed in the same room and same shift before their COVID-19 diagnosis was made. Viral sequencing performed on 4/5 patients' swabs showed no phylogenetic link between their viruses. The fifth patient (whose virus could not be sequenced) was dialyzed at the end of the dialysis room and was treated by a different nurse than the one in charge of the other patients. Three nurses shared the same virus detected in both self-care patients (one of them had been transferred to our in-center facility). The epidemiologically strongly suspected intra-unit cluster could be ruled out by viral genome sequencing. The infection control policy did not allow inter-patient contamination within the HD facility, in contrast to evidence of moderate dissemination within the nursing staff and in the satellite unit. Epidemiologic data without phylogenetic confirmation might mislead the interpretation of the dynamics of viral spreading within congregate settings.

KEYWORDS

epidemiology, genetics, genetic mapping, horizontal transmission, SARS coronavirus, virus classification

Abbreviations: HCW, healthcare worker; HD, hemodialysis; RT-qPCR, real-time reverse transcription-polymerase chain reaction.

Laura Labriola and Jean Ruelle contributed equally to this work.

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1 | INTRODUCTION

In-center maintenance hemodialysis (HD) patients are at potentially high risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (coronavirus disease 2019 [COVID-19]) by cross-contamination during HD sessions and travels to and from HD units. Furthermore, dialysis patients are particularly vulnerable due to their high burden of comorbidities, uremia-associated immune dysfunction, and older age.^{1,2} Indeed, in the early pandemic period, the incidence of COVID-19 in maintenance HD patients from 65 centers in Wuhan ($n = 7154$), China, was reported to be higher compared with the local general population (2% vs. 0.5%, respectively).³

Although various preventative policies to avoid the dissemination of SARS-CoV-2 have now become standard of care in hospital settings, clusters can occur despite robust infection control measures.⁴ We recently reported the evolution (over 3 months) of anti-SARS-CoV-2 antibodies in a cohort of adult, in-center chronic HD patients.⁵ We now assess the dynamics of SARS-CoV-2 transmission during the same early pandemic period in this carefully studied cohort and in staff members of the same HD facility. We used a phylogenetic approach, based on viral whole-genome sequences.

2 | METHODS

2.1 | Setting and preventative policies

Our in-center HD facility is located at the Cliniques Universitaires Saint-Luc (the main teaching hospital of UCLouvain, 983 beds), Brussels, Belgium. The unit has two shifts per day (up to 31 patients per shift), 6 days a week. At the time of this study, 98 patients were on maintenance HD in our facility (aged 68.8 [±14] years, 58% males, 47% diabetics).

The COVID-19 preventative protocol implemented in two phases is described in Table S1. On March 9, 2020, we became aware through personal contacts of the first cases of COVID-19 in Belgian HD units, soon followed by an outbreak in one of these units. We thus implemented in our unit on March 13 a protocol aiming at the early diagnosis and immediate isolation of HD patients infected with SARS-CoV-2. Our initial protocol (Table S1A) was implemented almost a month before but was largely consistent with the recommendations of the Centers for the Disease Control and Prevention (CDC), issued on April 12.⁶ As soon as a first nasopharyngeal swab tested positive by quantitative reverse-transcription polymerase chain reaction (RT-qPCR) for SARS-CoV-2 (Patient 1, March 20), the preventative policy was intensified, with the aim to avoid viral spread from any potentially infected individual (Table S1B). This intensified protocol particularly stressed the use of surgical facemasks by all staff members and all HD patients as long as they are in the facility.

As soon as they were diagnosed SARS-CoV-2 positive, patients were moved for their dialysis sessions to the dedicated COVID-19 isolation room (two shifts on the same day, three times a week). This room is usually reserved for either carriers of HBsAg or patients with

protective anti-HBs. At the end of each day with sessions of COVID-19 patients, this room (including chairs and all other surfaces) was disinfected by vaporization of a 6% hydrogen peroxide solution (Nocolyse Oxy'Pharm),⁶ allowing its safe use for COVID-19 positive (none of which was HBV+) and COVID-19 negative patients on alternate days. The three HBsAg-carrier patients, all COVID-19 negative, were transferred to another isolation room.

2.2 | SARS-CoV-2 real-time RT-qPCR

A nasopharyngeal swab for RT-qPCR testing for SARS-CoV-2 was performed in the following cases: (i) in case of symptoms suggestive of COVID-19^{5,6}; (ii) on April 6 or 7, systematically in all as yet negative HD patients.

SARS-CoV-2 RNA detection was performed as previously described⁷ using COVID-19 genesig[®] Real-Time RT-qPCR assay (Primerdesign Ltd) on a LightCycler 480 instrument (Roche Diagnostics). Primers and probes of this assay target the RNA-dependent RNA polymerase (RdRp) gene. A test with a cycle threshold less than 40 was considered positive.

2.3 | SARS-CoV-2 sequencing

Nasopharyngeal swabs were conserved in 1.5 ml of Universal Transport Media at -80°C until sequencing. RNA was extracted from 140 μl of the transport media of the specimen using the QIAamp viral RNA mini kit (QIAGEN). Fifty ng of purified RNA were retro-transcribed using the Maxima H minus double-stranded cDNA synthesis kit (Thermo Fisher Scientific) following the manufacturer's recommendations. A DNA NGS-library was then prepared using the Nextera DNA Flex for enrichment library kit (Illumina). Briefly, 50–1000 ng of cDNA are restricted in small fragments to create a DNA library of approximately 350 nucleotides long using bead-linked transposomes (eBLT) (Illumina). That tagmentation process is followed by a DNA clean-up and an amplification using unique-dual primers (IDT for Illumina UDI set of primers) recognizing the sequence added to each DNA strand during the tagmentation phase and adding a specific sequence to all strands of each sample. Amplified libraries were purified and dosed in spectrophotometry. Libraries are then pooled equimolarly and hybridized with a capture probe panel targeting SARS-CoV-2 as well as 40 other common respiratory viruses (Respiratory Virus Panel v2, Illumina). The enriched pooled library was finally amplified and purified. After a spectrophotometric dosage, the final library was diluted to 100 picomolar and charged on an iSeq. 100 cartridge allowing up to 8 million paired-end reads, in a 2×150 bp format.

2.4 | Sequences analysis

The on-board iSeq sequence analysis software generated the Fastq files, where reads were trimmed for primers and indexes sequences. Fastq files

were uploaded on the cloud-based ASP-IDNS[®]-5 analysis software (SmartGene). Analysis was made using the "beta-Coronavirus pipeline" version 2.2.0_COV_v0.2.

Briefly, paired-end reads were generated and automatically filtered for low-quality sections. The resulting reads were mapped against the SARS-CoV-2 profiles and mutations were detected in a quantitative manner (% reads aligned). A consensus genome was generated using a 40% cut-off for base determination and a minimal number of 30 reads per position.

Nextclade Beta version 0.8.1 was used as a first sequence aligner, allowing comparison to other documented SARS-CoV-2 strains and clade assignment (<https://clades.nextstrain.org>).

2.5 | Phylogenetic tree

The 22 sequences were aligned with CLUSTAL O (1.2.4) and 5'- and 3'- ends not included in the consensus alignment were trimmed: sequences used for the tree shared the same length of 29770 nt. Fasta files were then submitted to the NGPhylogeny web interface.⁸ The workflow included: sequence alignment using the MAFFT software,⁹ curation of the sequences with the block mapping and gathering with entropy (BMGE) software,¹⁰ tree generation using the fast distance-based phylogeny inference program FastME 2.0,¹¹ and tree output formatted with the Newick display.¹² Felsenstein's bootstrap analysis (not shown) was not informative given the very low diversity in our data set with some identical sequences' clusters.

2.6 | GISAID sequences accession numbers

Whole-genome sequences analyzed here were submitted to the GISAID platform and are accessible through the following identifiers: EPI_ISL_949244 to API_ISL_949250 and from EPI_ISL_1029958 to EPI_ISL_1029972.

2.7 | Outcome

The aim of our study was to identify the viral strains circulating in our unit, to understand the modalities of the spread of SARS-CoV-2

among patients and staff members within the facility (including potential clusters).

2.8 | Ethical approval

The study was performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki. The study protocol was approved by the Biomedical Ethics Committee of the Cliniques Universitaires Saint-Luc and UCLouvain Faculty of Medicine, Brussels, Belgium (protocol numbers 2020/11MAI/268 and 2020/29MAI/301).

3 | RESULTS

3.1 | Dynamics of SARS-CoV-2 transmission: The cases

On March 19, a 77-year-old man (Patient 1) had cough and fever during his HD session and was the first positive patient by RT-qPCR. During the next 18 days, seven additional symptomatic patients were positive by RT-qPCR: on March 19 (Patient 2), March 26 (Patients 3 and 4), March 28 (Patient 5), April 2 (Patient 6), and April 6, 2020 (Patient 7) (Figure 1, timeline). All of them lived at home. All negative patients ($n = 90$) were screened on April 6 and 7 by RT-qPCR and two asymptomatic patients (Patients 9 and 10), both nursing home residents, were found to be infected by SARS-CoV-2. Positive and negative patients are compared in Table 1.

Besides those 10 positive patients, two patients hemodialyzed in our self-care unit "Carpe Diem" were diagnosed on March 20 and 23 (Patients 11 and 12, respectively).

Patients 1 and 7 died from mesenteric ischemia and heart failure, 34 and 3 days after symptoms onset, respectively. Both patients had a very limited life expectancy before COVID-19 due to severe comorbidities.

Six out of the 10 positive patients usually came to HD sessions at the in-center unit on their own, that is, by car -alone or with a member of their family- or taking the metro (Patients 1-6). Despite our recommendations (Table S1), the remaining four patients (Patients 7, 8, 9, and 10) were transported with another HD patient in the same car, before their COVID-19 diagnosis, by a professional driver. However, none of these 4 patients transported with a

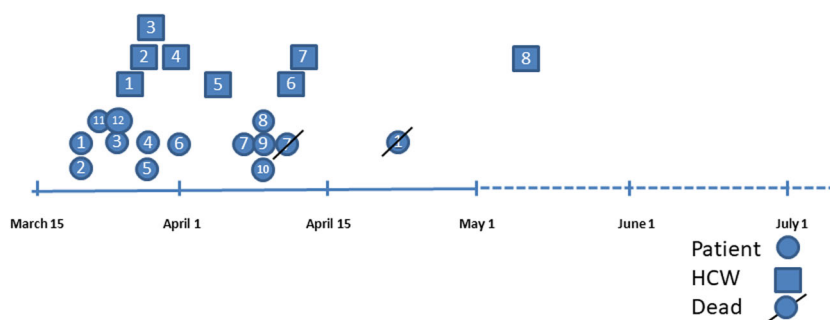


FIGURE 1 Timeline showing the dynamics of COVID-19 in HD patients and members of staff in our facility. COVID-19, coronavirus disease 2019; HD, hemodialysis

TABLE 1 Characteristics of HD patients diagnosed SARS-CoV-2 positive by RT-qPCR testing versus those uninfected

	Positive PCR n = 10	Negative PCR n = 88	p-value
Age, median (IQR), years	72 (61–77)	71 (62–79)	0.78
Male gender – no. (%)	5 (50)	51 (58)	0.63
Ethnicity			0.59
Caucasian – no. (%)	8 (80)	76 (86)	
Sub-Saharan African – no. (%)	2 (20)	12 (14)	
Diabetes – no. (%)	2 (20)	41 (47)	0.11
Vascular access			0.84
AV fistula – no. (%)	5 (50)	47 (53)	
Tunneled catheter – no. (%)	5 (50)	41 (47)	
HD vintage, median (IQR), months	68 (46–84)	34 (15–68)	0.03

Abbreviations: HD, hemodialysis; IQR, interquartile range; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

COVID-19 patient ever had symptoms suggestive of SARS-CoV-2 infection nor a positive RT-qPCR result. None of the drivers transporting one of these four PCR-positive patients experienced symptoms compatible with COVID-19.

Six nurses and one assistant nurse (out of 58 healthcare workers [HCWs]) had symptoms suggestive of COVID-19 and were positive by RT-qPCR between March 26 and April 15. One additional assistant nurse had a positive result on May 9 (Figure 1, timeline). All of them recovered without hospitalization.

As depicted in Figure 2, five positive patients (Patients 2, 4, 5, 6, and 10) were usually dialyzed in the same room in the morning shift on Tuesday, Thursday, and Saturday, before their COVID-19 diagnosis was made. This room includes eight chairs. Patients 2 and 5, and Patients 6 and 10 were usually dialyzed in contiguous places, separated by 1.6–1.7 m. Thus, an intra-unit cluster was strongly suspected. The other patients (Patients 1, 3, 7, 8, and 9) were dialyzed at stable chairs, in different rooms, and/or in different shifts (Figure S1a–c).

3.2 | Phylogenetic analysis

To explore the possibility of a cluster, we performed whole-genome sequencing analysis on the diagnostic RT-qPCR samples. As some samples had a low viral load (high Cq values in RT-PCR), a sequence could only be obtained in 7 out of 12 positive patients (Patients 2, 5, 6, 8, 10, 11, and 12), and in 6 of them, the entire genome could be sequenced (Patients 2, 6, 8, 10, 11, and 12). Five other sequences could be obtained from members of the nursing staff of the HD unit (HCWs HD 1–5). Ten sequences obtained from patients hospitalized

at the same institution but not on HD served as the control group. Table S2 details the clade and the mutation associated with each sequence, and Figure 3 shows the phylogenetic relationships.

The samples belonged to various lineages, frequently found in Belgium during the first wave of the pandemic, such as B.1.6, B.1.83, or B38, as detailed in Table S2. We found no direct phylogenetic link between the viral strains of Patients 2, 5, 6, and 10, that is, four out of the five positive patients belonging to an epidemiological cluster. For Patient 5, the genomic sequence was incomplete, however, the coverage was sufficient to classify his strain in clade 20C/B.1 which is a different strain from those carried by Patients 2, 6, and 10. RT-qPCR sample from Patient 4 was not available; however, his chair was at the end of the dialysis room (Figure 2) and he was treated by a different nurse than the one in charge of Patients 2, 5, 6, and 10. This makes the occurrence of an intra-unit cluster very unlikely, suggesting a good efficiency of the preventative policy implemented after the diagnosis of the very first cases.

However, Patients 11 and 12, who both were in the same HD shift in our satellite self-care facility, share the same virus (Figure 3) classified as lineage B.1.83. Three HD nurses (HCWs HD 1, 2, and 4) also share the same virus, with only one point mutation difference compared to the virus of Patients 11 and 12. The timing of diagnosis is compatible with a transmission from Patient 11, transferred to our in-center unit, to one member of the nursing staff who cared for that patient (HCW HD1), followed by transmission within the staff, since HCW HD2 and HCW HD4 never were in charge of Patient 11.

One other HD unit sequence, from Patient 10, shares a possible phylogenetic link (only two-point mutations difference across the whole genome) with the sequence from control Patient 4, treated in another unit of the hospital, although no direct link between those patients could be established.

No additional SARS-CoV2 infection was found by systematic RT-qPCR screening performed on July 6 and 7 and November 5 and 6, 2020 in HD patients. Moreover, on January 20 and 21, 2021 serum samples from all patients were screened with a total anti-SARS-CoV-2 antibodies qualitative electro-chemiluminescent immunoassay using a recombinant nucleocapsid antigen (anti-SARS-CoV-2 N) (Roche Elecsys); no RT-qPCR negative patient was found to have developed SARS-CoV-2 infection-induced seroconversion.

4 | DISCUSSION

To the best of our knowledge, this is the first study using the gold standard viral genome sequencing to assess the modalities of the spread of SARS-CoV-2 in an in-center HD facility. This analysis shows that the epidemiological cluster (five positive patients on HD in the same room at the same time) is not confirmed by sequencing. Thus the infection control policy did not allow inter-patient contamination within the in-center HD facility, in contrast to evidence of dissemination within the nursing staff, during the very first pandemic phase. Admittedly, both our patient-to-patient transmission in the satellite self-care HD unit and transmission between staff members

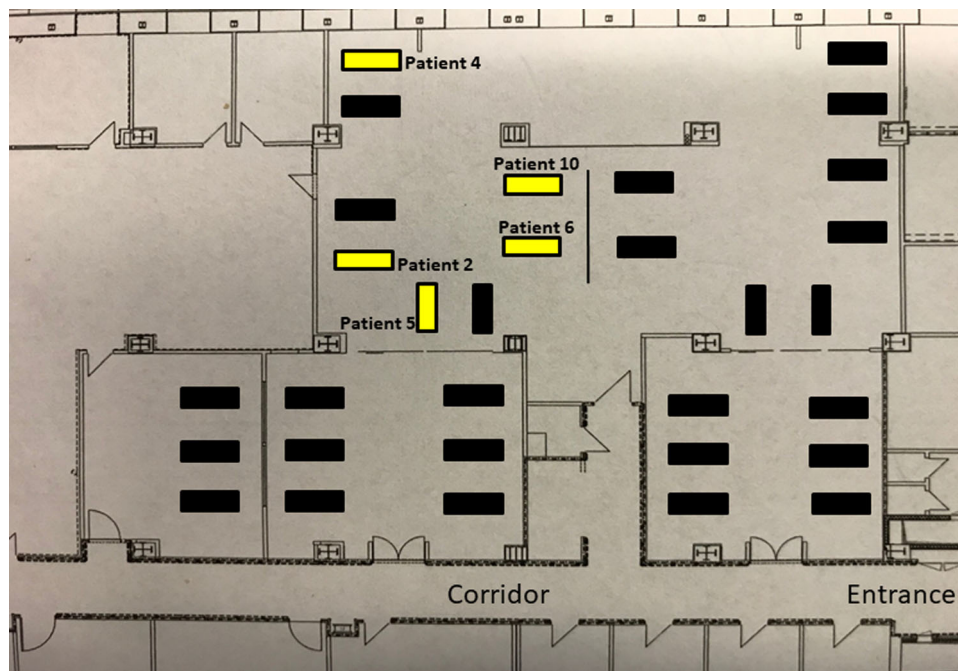


FIGURE 2 Full-scale map of the dialysis facility. Distance between two chairs: 1.6–1.7 m. Five COVID-19 patients (Patients 2, 4, 5, 6, and 10) were dialyzed at the same time (Tuesday, Thursday, and Saturday, morning shift) before being diagnosed by RT-qPCR. The other five COVID-19 patients were dialyzed on other days or shifts (Figure S1a–c). COVID-19, coronavirus disease 2019; qRT-PCR, quantitative reverse-transcription polymerase chain reaction

of the in-center unit demonstrate that transmission may occur and is detected by our molecular virology technique.

In our previous study, almost all (8/9) COVID-19 chronic HD patients developed specific antibodies within the first month after symptom onset.⁵ Interestingly, no RT-qPCR-negative patient developed SARS-CoV-2 infection-induced seroconversion in our unit, underlining the crucial role of the preventative measures implemented very early for both patients and staff in our HD unit as compared to other HD units which reported large outbreaks.^{13,14} Indeed, our intensified preventative protocol implemented on March 20 (simultaneously with the publication of the EUDIAL recommendations for COVID-19 pandemics¹⁵) included the mandatory wearing of surgical face masks before, during and after the HD session by all patients and staff members. This was implemented several weeks before a similar recommendation by the CDC, issued on April 12.⁶ Before this date, the CDC recommended face masks only for patients with symptoms suggestive of COVID-19. Importantly, data from a large American health care system have shown that universal masking of HCWs and patients was associated with a significantly lower rate of SARS-CoV-2 infection among HCWs, whereas the number of cases continued to increase in the general population, despite local and statewide measures.¹⁶

Admittedly, as the viral load in nasopharyngeal samples was too low in some patients (1, 3, 4, 7, and 9), a within-unit spread of SARS-CoV-2 from patient to patient cannot be completely ruled out (Figure 2). Yet, in that case, it was at most minor, as compared with many other reports. Furthermore, these patients whose virus could

not be sequenced were dialyzed in different rooms or different shifts, making this hypothesis very unlikely.

Interestingly in a study comparing aggregated daily counts of confirmed COVID-19 cases in the general population in the US and in patients on HD in Fresenius Medical Care North America units from March 1 to July 29, 2020, Cherif et al.¹⁷ found that SARS-CoV-2 spread (as indicated by the time-varying reproduction number) in HD patients mirrored the background transmission in the general population. However, this parameter declined earlier in the dialysis population, strongly supporting the benefit of preventative measures in reducing risk in HD facilities.

Seroprevalence in HCWs of our facility was 18.2%, well above the figures reported in 326 HCWs from COVID-19 positive units in a tertiary Belgian hospital (8.3%),¹⁸ and in 3255 HCWs from our own University hospital (7.8%),¹⁹ but less than the 35% reported by week 13 after the first diagnosed COVID-19 patient in a pediatric HD unit from Indianapolis.²⁰ Although these differences might be due to differences in the earlier or later antibody testing in the evolution of the epidemic, they emphasize the high-risk associated with HD, that is, care provided to patients coming repeatedly from outside the facility and underlines the utmost importance of preventative policies for patients and HCWs in HD units.

The main strength of this study is the investigation at the molecular virology level of the effectiveness of hygiene measures within an HD unit. Moreover, RT-qPCR screening was performed very early in all HD patients of the facility, regardless of symptoms or contacts with positive patients, allowing the identification of two

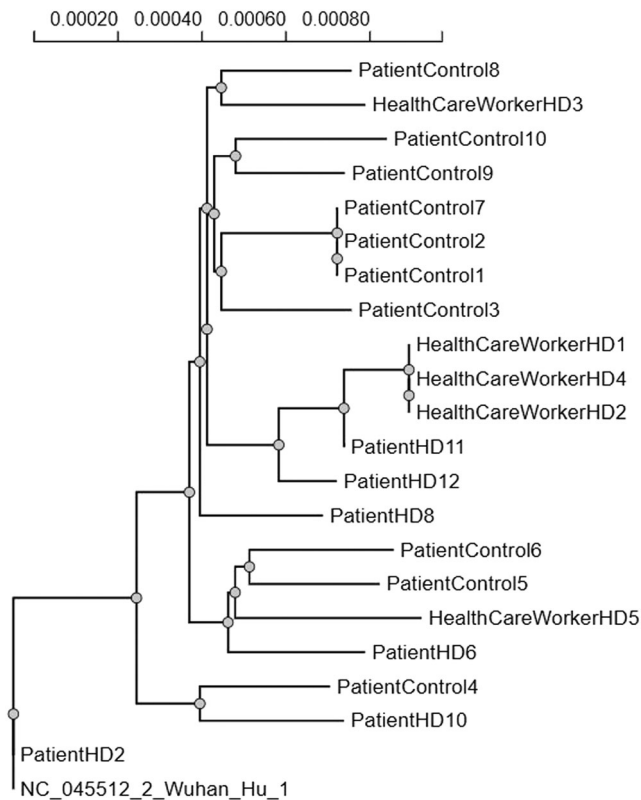


FIGURE 3 Phylogenetic relationships between 22 viral sequences. Six viral sequences are related to the patients treated in the in-center hemodialysis (HD) unit (Patient 2, Patient 6, Patient 8, Patient 10, Patient 11, and Patient 12), five sequences are related to the nursing staff of the HD unit (Health Care Workers HD1–HD5), 10 sequences are coming from control patients sampled during the same period in the Hospital but outside the HD unit (Patient Controls 1–10). NC_045512.2 is the reference strain originally sequenced in Wuhan, China

asymptomatic RT-qPCR positive patients. No additional SARS-CoV2 infection was detected by two additional RT-qPCR screenings performed in all HD patients. Additionally, no other RT-qPCR negative patient seroconverted as a result of COVID-19 infection until January 2021. Our results confirm that the preventive protocol implemented in our HD facility, modified after the first COVID-19 diagnosis, was highly effective as we found no clue of transmission between our in-center HD patients in our molecular study.

Our study has some limitations. First, it was performed in a single-center, and the number of patients included was relatively low, thanks to the preventative protocol. Second, the phylogenetic analysis showed a definite link between two patients from our self-care unit (one of them transferred to our in-center facility) and three members of the nursing staff of the in-center facility, but some links could be missed as some positive samples could not be sequenced due to low viral load. Indeed, a staff-to-patient transmission caused by an infected, asymptomatic HCW cannot be completely ruled out. However, the results of the phylogenetic analysis, available in most

patients and the fact that the patients not belonging to the cluster were dialyzed in different rooms (Patients 1, 7, and 8, and Patients 3 and 9) make the hypothesis of a direct patient-to-patient transmission very unlikely.

5 | CONCLUSION

Our results highlight the crucial role of phylogenetic virological analysis for the accurate diagnosis of an epidemiological cluster of SARS-CoV-2 infection within congregate settings. In our study, the strongly suspected intra-unit cluster among HD patients could be ruled out. Epidemiologic data without phylogenetic confirmation might mislead interpretation of the dynamics of the viral spreading.

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CONFLICT OF INTERESTS

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AUTHOR CONTRIBUTIONS

Research area and study design: Laura Labriola, Jean Ruelle, Benoît Kabamba and Michel Jadoul. Data acquisition: Laura Labriola, Jean Ruelle, Anaïs Scohy, François Seghers, Quentin Perlot, Christine Desmet, and Cécile Romain. Data analysis and interpretation: Laura Labriola, Jean Ruelle, and Michel Jadoul. Writing: Laura Labriola, Jean Ruelle, and Michel Jadoul. Supervision or mentorship: Michel Jadoul, Laura Labriola, Jean Cyr Yombi, Julien De Greef, Benoît Kabamba, and Hector Rodriguez-Villalobos.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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