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Editorial Comment

EDITORIAL COMMENT

Which criteria should we use to end isolation in hemodialysis patients with COVID-19?

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ABSTRACT

Safe and timely discontinuation of quarantine of in-center hemodialysis (HD) patients with a previous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a challenging issue for the nephrological community because current guidelines for ending isolation do not mention dialysis patients. To prevent potentially fatal outbreaks of coronavirus disease 2019 (COVID-19), a cautionary approach has been adopted by most dialysis units. The criteria for ending the isolation in the HD population generally coincide with those recommended for immunocompromised people. Thus, a test-based strategy relying on two consecutive negative reverse transcriptase-polymerase chain reaction (RT-PCR) nasopharyngeal swabs has been adopted to terminate quarantine. This strategy has the disadvantage of prolonging isolation as RT-PCR positivity does not equate to SARS-CoV-2 infectivity. Consequentially, prolonged positivity of SARS-CoV-2 results in excessive workload for the HD staff who must face an increasing number of COVID-19 patients requiring isolation. This condition leads also to serious implications for the patients and their households including work productivity loss, postponement of health-care appointments and an increased risk of COVID-19 reinfection. To counteract this problem, other diagnostic tests should be used to provide the best care to HD patients. Recent results seem to encourage the use of RT-PCR cycle threshold (Ct) values and rapid antigen tests given their better correlation with cell culture for SARS-CoV-2 than RT-PCR testing. Here, we provide an overview of the current scientific evidence on the tests used to verify the infectiousness of the virus in order to stimulate the nephrological community to adopt a streamlined and pragmatic procedure to end isolation in COVID-19 patients on HD.

Keywords: rapid antigen test, cell culture, COVID-19, hemodialysis, virus shedding, isolation, PCR Ct values, quarantine, RT-PCR, SARS-CoV-2, subgenomic RNA

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Ending the isolation of in-center hemodialysis (HD) patients with coronavirus disease 2019 (COVID-19) is a real-life dilemma for the dialysis staff who are entangled the labyrinth of up-to-date guidelines that do not include dialysis patients. Safe and timely discontinuation of isolation is key for ensuring proper infection control measures against COVID-19, especially within an 'enclosed' community such as the HD unit. Here, frequent face-to-face interactions between vulnerable patients and health-care workers make the dialysis unit a place prone to regular outbreaks, which may have serious consequences for the dialysis population [1].

Recently, the spread of the highly mutated Omicron variant has renewed the wide array of challenges for the dialysis staff involved in preventing the diffusion of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Stress on the HD units has been substantial and probably higher than on other health-care facilities given that the delivery of HD care is not amenable to modified treatment schedules such as appointment postponement or telemedicine. Additionally, the impossibility of expanding the HD treatment area to separate COVID-19 patients and contacts from uninfected patients has driven a deep organizational change in HD treatment schedules. For instance, our dialysis center experienced a reorganization of the dialysis shift as about 13% of the in-center HD patients simultaneously required quarantine in dedicated isolation rooms during the Omicron peak outbreak. In parallel, a high rate of breakthrough infections among dialysis staff contributed to a significant personal shortage as about 10% of the nurses became ill in the same period.

Beyond the surge of cases and the infrastructure limits of the dialysis units, the prolonged viral shedding measured by RT-PCR on nasopharyngeal swabs is appraised as a further cause of workplace stress because it tends to increase the cumulative number of cases requiring quarantine [2].

There is a substantial agreement on the end of isolation of HD patients. According to data that are derived from respiratory viral transmission studies in the general population, the releasing of COVID-19 HD patients from isolation is based on two negative reverse-transcriptase chain reaction (RT-PCR) nasopharyngeal swab results on sequential samples taken at least 24 h apart [3–5]. Studies conducted on the dialysis population documented that isolation of COVID-19 patients generally lasts 34–44 days from symptom onset to the first negative RT-PCR test [6–8], although a longer viral shedding has been reported in anecdotal cases [9, 10]. However, a growing body of evidence suggests that RT-PCR nasopharyngeal swab is associated with a prolonged positivity [9, 11] that may not reflect the infectivity of the virus [12, 13]. Sporadic positive RT-PCR results may be obtained many months after the initial infection, even with multiple negative results in the interim since detectable SARS-CoV-2 RNA is generally found in upper respiratory specimens for up to 3 months after illness onset [14]. This issue has been largely addressed by the scientific literature, even though most of the published studies, conducted on patients not receiving dialysis, yielded conflicting information on the infectiousness of the SARS-CoV-2. Data conducted on the general population showed that patients with mild COVID-19 had no viable SARS-CoV-2 on sputum, pharyngeal swabs and stool after 8 days from the onset of the symptoms despite high viral loads measured by RT-PCR on the same specimens [15]. Similar findings have been documented in hospitalized patients with severe COVID-19, where the median duration of shedding infectious virus using cell culture for SARS-CoV-2 was confirmed at 8 days from onset of symptoms [16]. Another study reported instead that about one-third of mildly symptomatic immunocompetent patients with long-lasting positive RT-PCR ($\geq$14 days) on nasopharyngeal swabs tested positive for SARS-CoV-2 isolation in cell-based culture [17]. A systematic review and meta-analysis revealed that SARS-CoV-2 shedding duration (measured by RT-PCR) was 17 and 14.6 days in the upper and lower respiratory tract, respectively, whereas the duration of the viable virus was shorter (up to a maximum of 8 days from symptoms onset) [18]. However, a longer shedding of the infectious virus has been observed even in healthy subjects with long-lasting COVID-19 manifestations [19].

Considering the HD patients as moderately or severely immunocompromised subjects, the current guidance of the US Centers for Disease Control and Prevention (CDC) suggests for this group ending isolation at least 20 days after symptom onset in conjunction with a test-based strategy consisting of two negative results from at least two consecutive respiratory specimens collected $\geq$24 h apart using an antigen or an RT-PCR test [20]. Recently, the European CDC implemented the test-based strategy with a time-based criterion, in which the end of isolation of immunosuppressed patients may occur 20 days after the onset of symptoms without any confirmation test [21].

Based on these recommendations, two questions were raised about the management of in-center HD patients:

(i) Are HD patients a vulnerable subset of the population?
(ii) Which method should be used to terminate isolation in HD patients?

With regard to the first query, it is widely accepted that HD patients have a dysfunction of the immune system involving the main branches of the immune system, innate and adaptive immunity [22]. The immune dysfunction is induced by the uremic molecules or by dialysis treatment itself [23]. HD patients have a higher risk of infections [24] and lower response to the vaccine [25] compared with the general population. For this reason, a great emphasis was placed on the prioritization of the COVID-19 vaccination for HD patients [26]. Patients receiving HD showed that responsiveness to COVID-19 vaccination was substantially delayed for both the humoral- and cell-mediated branches of the immune response [27]. Although response rates after the second dose (89%) were almost comparable to healthy controls [28], antibody levels were significantly lower [29]. A failure to develop an immune response occurred in patients with a low *Kt/V* for urea and with immunosuppressive therapy [30]. It is clear that HD patients should be theoretically considered at least as moderately immunosuppressed subjects and de-isolation should be cautious and take into consideration a longer viral shedding compared with the general population. However, the severity of COVID-19 cannot be overlooked. Patients who remain asymptomatic or mildly symptomatic (no fever) represent a different subset of the population and should not be assimilated into patients with a severely defective immune system.

For the second question, the end of isolation should coincide with the clearance of active virus replication in the upper respiratory tract in order to prevent SARS-CoV-2 transmission among HD patients. The tests used to assess the end of isolation are cell culture for SARS-CoV-2, and molecular and rapid antigen tests. SARS-CoV-2 culture is the best method to establish, on a real-time basis, the viability of SARS-CoV-2 on nasopharyngeal samples. This multi-step process includes inoculation of the biological specimen on cells harboring SARS-CoV-2 (e.g., Vero cells) with the addition of growth media, antibiotics and antifungal drugs, incubation of the plates for 5–7 days, recognition of cytopathic effects and identification of SARS-CoV-2 by immunofluorescence or RT-PCR assays. This procedure is performed only

Table 1. Advantages and disadvantages of strategies used for ending isolation in COVID-19 patients

Strategy	Pros	Cons
Test-based method[a]		
RT-PCR qualitative assay	• High sensitivity	• Poor correlation with infectiousness • Equipment • Qualified personnel • Cost • Slow analytical process
RT-PCR quantitative assay	• Quantification of the viral load	• Unknown relationship between viral load and infectiousness of SARS-CoV-2 • Need for RT-PCR instrument
RT-PCR Ct values	• Good correlation with infectiousness • Parameter routinely assessed by RT-PCR assay	• Risk of residual infectiousness • Need for RT-PCR instrument
RT-PCR subgenomic RNA	• Good correlation with infectiousness	• Limited data on its diagnostic usefulness • Restricted use in research projects • Need for RT-PCR instrument
Rapid antigen test	• High specificity • Widely accessible • Easy to operate • Cost-effective • Fast analytical process	• Low sensitivity in asymptomatic patients
Cell-culture of SARS-CoV-2[b]	• Gold-standard	• Variability of SARS-CoV-2 replication in different cell lines • Adequate structure • Equipment • Time-consuming • Qualified personnel • Cost
Time-based strategy	• Cost-effective • No need for equipment and qualified personnel	• Residual risk of infectiousness especially in severely immunosuppressed, frail and critically ill patients
Symptoms-based strategy	• Cost-effective • No need for equipment and qualified personnel	• Residual risk of infectiousness • Risk of prolonging quarantine in patients with symptoms of long COVID • Impossibility to establish quarantine in asymptomatic patients

[a]Results of RT-PCR analysis and SARS-CoV-2 culture may be subject to inter-laboratory variability
[b]African green monkey kidney cell line 'Vero cell' harbors high levels of SARS-CoV-2 replication. Other cell lines such as human Calu-3 (non-small-cell lung cancer cell line) and CaCo-2 (colorectal adenocarcinoma cell line) support higher level of SARS-CoV-2 replication than human epithelial cells in the upper and lower respiratory tract.

in 'Biosafety Level 3' facilities and needs a labor-intensive and time-consuming process; therefore, it is not practicable in the majority of the laboratories.

Molecular diagnostic to detect SARS-CoV-2 is based on RT-PCR, which includes a qualitative, semi-qualitative and quantitative assay. Qualitative RT-PCR assay is an extremely sensitive technique useful to detect SARS-CoV-2 genetic material in tissue samples. Generally, RT-PCR targets two or more genes to increase the sensitivity and specificity of the technique. Test results can be 'detected' (i.e., positive), 'undetected' (negative) or 'indeterminate/inconclusive' if only one of the two or more gene targets has been detected.

A semi-quantitative assay [cycle threshold (Ct)] can be assessed by RT-PCR without any additional efforts in terms of cost, time and workforce. RT-PCR Ct value is a practical method to indirectly measure SARS-CoV-2 viral load. Ct value is the number of cycles at which fluorescence of the RT-PCR product is detectable over and above the background signal. This measure is inversely proportional to the amount of RNA in the sample and higher Ct values generally correlate with low viral load, which, in turn, correlates with a decreased infectiousness [31]. High Ct values and clinical resolution of the disease seem to predict resolution of the infection. Bullard *et al.* [32] showed that a Ct value >24 and duration of symptoms >8 days are indicative of reduced SARS-CoV-2 infectivity in the general population. In this study, Ct value >24 showed a diagnostic specificity, namely, the proportion of noninfectious samples, of 97%. Similar results showed that a Ct value >34 has been considered no longer contagious [33]. A pragmatic approach, recently divulged by US authors, consists of discontinuing quarantine when the viral load is below 100 000 copies/mL, corresponding to a Ct value of >28–31 [29]. However, high Ct values do not exclude a little residual risk of infectivity [15].

Detection of subgenomic SARS-CoV-2 RNA through RT-PCR is another surrogate of active SARS-CoV-2 infection [34]. Subgenomic RNAs are smaller sequences than genomic RNA.

They are the product of a unique mechanism of coronavirus transcription encoding structural proteins of the virus (i.e., spike, membrane, envelope and nucleocapsid protein) during the intermediate or later stage of the infection. Subgenomic RNAs are only transcribed in infected host cells and are not packaged into SARS-CoV-2; therefore, it is thought to better reflect replication-competent virus than nonreplicating virus. A recent study confirmed these impressions and showed that the mean duration of positive SARS-CoV-2 culture (11.39 ± 10.34 days) after symptoms onset was not statistically significantly different from subgenomic RNA detection (13.75 ± 11.22 days). The duration of positive SARS-CoV-2 culture was instead significantly shorter than genomic RNA shedding (22.85 ± 11.83 days) [35].

RT-PCR quantitative assay amplifies the target genetic sequence and furnishes the concentration of that DNA species. This procedure is essential for viral load determination that has been reported as a determinant of severity of illness [36] and virus transmissibility [37]. Van Kampen *et al.* [38] documented that the probability of isolating infectious SARS-CoV-2 was extremely low when the viral load was below 6.63 Log10 RNA copies/mL. However, the lack of a clear relationship between the viral RNA load threshold and ineffectiveness of the virus limits, for now, the application of RT-PCR quantitative assay to end isolation in our patients.

Lastly, rapid antigen test, recently approved for the diagnosis of COVID-19, could be a promising test to establish the persistence of viable viruses on the mucosa of infected patients [39]. Evidence suggests that authorized antigen-based testing may align better with SARS-CoV-2 culture-based test results than RT-PCR. A recent study evaluated RT-PCR assay and rapid antigen test of SARS-CoV-2 culture, in upper respiratory specimens from 251 participants. Surprisingly, the SARS-CoV-2 antigen test had an excellent performance profile compared with viral culture, the reference method to assess effectiveness. The positive percentage agreement for detection of infectious virus for the rapid antigen test (96.4%) was similar to RT-PCR (100%) when these two tests were compared with culture results. Rapid antigen test also showed a positive predictive value of 90.0%, whereas the RT-PCR assay showed a positive predictive value of only 73.7% [40]. More importantly, the negative predictive value of the rapid antigen test (99.5%) can potentially improve infection control measures and reduce the duration of quarantine in subjects with prolonged RT-PCR positivity [40].

According to recent evidence, PCR assay provides false-positive results because it targets noninfectious genetic material, slowly degraded by the host immune system, on the nasopharyngeal mucosa [39]. This phenomenon, common to other viral infections (SARS-CoV, Middle East respiratory syndrome coronavirus, influenza virus, Ebola virus, Zika virus and measles virus) results in unnecessarily prolonged isolation without any advantage for the patients and the dialysis staff [39]. Ideally, de-isolation processes should provide timely release of the COVID-19 patient without any risk of virus spreading within the dialysis unit. Actually, the vulnerability of the HD population, the burden of potential errors along the analytical chain process of the current tests and the limitations of the current strategies (Table 1) make de-isolation of COVID-19 HD patients an extremely challenging task, as wrongly interpreting a positive PCR result can have severe implications for the safety of HD patients. Furthermore, the lack of studies on the diagnostic accuracy of these tests in the dialytic population should suggest a cautious approach. It is of paramount importance that the physician interprets the test results on a case-by-case basis by keeping in mind the clinical information of the patient and the stage of the illness. In the absence of information on the stage of the infection, re-testing for a second RT-PCR test (Ct value, quantitative assay) allows minimization of the risk of preanalytical errors and—even more importantly—tracing of the SARS-CoV-2 dynamic. In this case, a higher Ct value suggests a past COVID-19 infection whereas a lower value reflects an underlying infection during the incubation period.

The principal advantages of this paradigm shift may be a reduction of pressure on the HD unit and the decreased risk of SARS-CoV-2 reinfection during the prolonged quarantine. Furthermore, a timing procedure of de-isolation may ease the process of patient transfer between facilities and solve the weird paradox of being managed as COVID-19-infected patients within the dialysis unit and living a normal life outside the dialysis center, according to the international guidelines concerning the use of the rapid antigen test.

In conclusion, the new insights suggest that RT-PCR nasopharyngeal swab is not indicative of infectivity after recovery from COVID-19. Evidence based–science criteria are urgently required to streamline the procedure of de-isolation of in-center HD patients to avoid reinfections, excessive cost, reduce pressure on the healthcare system as well as delivery suboptimal care for this group of patients. Given the limited access to SARS-CoV-2 culture in most laboratories, rapid antigen test and RT-PCR Ct values (or a combination of both) are so far promising and accessible surrogates of SARS-CoV-2 replication, especially in HD patients with asymptomatic or mild symptomatic COVID-19.

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CONFLICT OF INTEREST STATEMENT

None declared.

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