Renal Replacement Therapy

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# Prolonged RT-PCR test positivity in hemodialysis patients with COVID-19

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## Abstract

**Background** The weakened immune system of patients on hemodialysis (HD) may prolong SARS-CoV-2 infection compared to the general population. Current international guidelines recommend ending isolation in conjunction with serial testing in moderately and severely immunocompromised subjects. This study aimed to estimate SARS-CoV-2 infectivity by measuring RT-PCR test positivity in HD patients. A comparison between RT-PCR test and cycle threshold (Ct) value has been performed as a secondary endpoint.

**Methods** A single-center retrospective study was conducted at the University of Modena (Italy) from March 2020 to October 2022. Only patients on chronic HD therapy with COVID-19 were enrolled in the study. In our HD Center, two negative nasopharyngeal reverse transcription polymerase chain reaction (RT-PCR) results were used to end quarantine in this population. SARS-CoV-2 RT-PCR test positivity duration measured the time elapsed from a positive RT-PCR to a second negative test. Ct cut-off of 35 cycles was used to definite "high Ct value," a condition characterized by a large number of cycles of PCR amplification to register a positive RT-PCR test.

**Results** During the observational period, 159 cases of SARS-CoV-2 infections were diagnosed in 151 patients. Median age was 70.1 (54.3–81.6) years and males accounted for 59.6% of the COVID-19 population. Median duration of SARS-CoV-2 RT-PCR test positivity on the nasal mucosa accounted for 30 (IQR, 21–40.5) days. Unvaccinated patients experienced significantly longer RT-PCR test positivity compared to vaccinated patients (42 [IQR,31–56] vs. 28 [IQR,20–35.7] days; $p = < 0.001$). The use of high Ct value, a laboratory surrogate of SARS-CoV-2 replication, anticipated a negative RT-PCR test of 9 (IQR, 6–12) days. Multivariate linear regression analysis showed that increased age ($\beta$ coefficient 0.31; confidence interval [CI] 95%, 0.14—0.43; $p = < 0.001$) and the lack of anti-SARS-CoV-2 vaccination ($\beta$ 0.49 CI95%, 11.9–22.5; $p = < 0.001$) were predictors of a prolonged RT-PCR positivity.

**Conclusions** Patients with COVID-19 on HD had prolonged RT-PCR test positivity. The adoption of "high Ct value" criteria led to a significant reduction in the duration of RT-PCR test positivity compared to the use of the classical nucleic acid amplification test. In our study, the lack of SARS-CoV-2 vaccination and older age were independently associated with a longer RT-PCR positivity.

**Keywords** COVID-19, Vaccine, Mortality, SARS-COV-2, Virus shedding, Ct value, RT-PCR, Hemodialysis, Virus hesitancy, Hospitalization

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## Introduction

Patients on hemodialysis (HD) are at high risk of experiencing severe consequences from COVID-19 [1] because of the burden of comorbidities [2–4] and weakened immune systems [5]. In addition, these patients are at high risk of being exposed to the virus spread by other patients and healthcare workers during dialysis treatment [6]. As a result, a series of containment measures have been adopted by the dialysis staff to limit SARS-CoV-2 transmission among HD patients. Isolation of COVID-19 patients until virus clearance has been one of the most highly prioritized measures within the dialysis units to protect uninfected patients and dialysis staff from COVID-19 patients [7, 8].

Experience gained during these three years of COVID-19 pandemic points out the challenging management of HD patients, as they require a longer duration of isolation compared to the general population due to their prolonged RT-PCR test positivity, generally exceeding 20 days [9–11]. For moderately and severely immunosuppressed patients, the current Centers for Control and Prevention (CDC) guidelines recommend ending isolation using a test-based strategy, which confirms the clearance of the virus [12].

From the beginning of the pandemic, two consecutive negative RT-PCR or rapid antigen test results, ideally with a minimum of a 24-h interval, have been used for the discontinuation of isolation in this subset of the population [13, 14]. Recently, cycle threshold (Ct) value has been implemented in clinical practice to assess the replication of SARS-CoV-2. As the classical RT-PCR test is unable to reflect virus infectivity, Ct value might distinguish a false test positivity from virus replication without any additional efforts in terms of cost, time and workforce. Ct value indirectly measures SARS-CoV-2 viral load by indicating the number of cycles at which the RT-PCR signal is detectable over and above the background levels. Over the last months, there has been growing enthusiasm for the potential usefulness of Ct values, albeit these values lack standardization among RT-PCR instruments. High Ct values predict the resolution of the infection by anticipating the negativity of classical RT-PCR. Indeed, classical nucleic acid amplification test may prolong SARS-Cov-2 infectivity by detecting fragments of RNA of non-viable viruses [15].

In our Center, to shield our patients and prevent COVID-19 outbreaks, we maintained an active program of screening for asymptomatic patients and ended quarantine until two negative RT-PCR tests for all HD patients with COVID-19 from the beginning of the pandemic. This study aimed to widen the knowledge of the COVID-19 in HD population by evaluating the duration and predictors of SARS-CoV-2 RT-PCR test positivity. The implementation of Ct values in the diagnostic armamentarium of SARS-CoV-2 infectivity spurred us to evaluate the performance of this new test. A comparison of the duration of viral RNA positivity between Ct value and classical nucleic acid amplification test has been performed as a secondary endpoint of the study.

## Materials and methods

A single-center retrospective analysis was conducted on 312 patients on chronic HD therapy followed at the University Hospital of Modena, Italy, from March 1, 2020, to October 31, 2022. Only HD patients with a diagnosis of COVID-19 (n. 151) were enrolled in the study. For participants who experienced multiple episodes of SARS-Co-2 infections, we considered only patient's clinical and demographic characteristics of the first episode. COVID-19 diagnosis was performed through RT-PCR nasopharyngeal swab. Duration of SARS-CoV-2 RT-PCR test positivity was evaluated only in survivors and measured the time elapsed from diagnosis of COVID-19 to the second negative nasopharyngeal RT-PCR test. Positive COVID-19 patients were monitored by RT-PCR nasopharyngeal swab once a week until the first negative test result, and every dialysis session thereafter. To avoid heterogeneity arising from different diagnostic tests used to end the isolation of COVID-19 patients, the results of antigen tests, used to screen patients periodically, were not considered to estimate the duration of SARS-CoV-2 RT-PCR test positivity. In our Center, the screening of all asymptomatic HD patients was performed using a chemiluminescence immunoassay (CLIA) technology for the quantitative detection of SARS-CoV-2 nucleocapsid antigen protein. Rapid antigen test screening was performed bimonthly from January 2021 to December 26, 2021, and weekly from December 27, 2021. Before dialysis entry, body temperature, masking, COVID-19 symptoms and handwashing using alcohol-based hand sanitizer were checked by a dialysis nurse. All antigen-positive tests were confirmed within a few hours through nasopharyngeal swab RT-PCR testing. For patients with symptoms or contact of COVID-19 patients, RT-PCR testing was the test of choice.

This cohort of patients was subdivided into unvaccinated and vaccinated groups. The term "unvaccinated" refers primarily to patients who contracted SARS-CoV-2 infection before the availability of mRNA vaccine or patients that refused vaccination, whereas the term "vaccinated" refers to patients who completed the vaccination cycle according to international guidelines at the time of COVID-19 diagnosis. We excluded from the study population unvaccinated patients with SARS-CoV-2 reinfection in order to exclude immunized COVID-19 patients in the unvaccinated group.

RT-PCR Ct value was available only from March 2022. Ct value greater than the cutoff of 35 cycles of replication was defined as "high Ct value." This condition reflects a low level of viral RNA replication and therefore a low probability of SARS-CoV-2 infectivity.

Immunosuppressed subjects included patients who underwent previous immunosuppressive therapy for autoimmune disease or kidney transplantation. Hospitalization refers to patients with severe illness (SpO2 < 94% on room air at sea level, fever, dyspnea, and lung infiltrates) who were admitted to the hospital to receive appropriate care. Anti-SARS-CoV-2 antibody response was measured from November 2021, when most of the population underwent third dose of mRNA vaccine.

The test (chemiluminescent immunoassay- CLIA [LiaisonVR, DiaSorin]) measured the level of serum anti-spike IgG. Two RT-PCR assays were used for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens: the Alinity *m* System SARS-CoV-2 kit (Abbott Molecular, Inc, Des Plaines, USA) and the Allplex SARS-CoV-2 Assay (Seegene, Seoul, Korea). The Alinity *m* SARS-CoV-2 assay detects N and RdRP genes of SARS-CoV-2, whereas Allplex SARS-CoV-2 Assay detects N, S and RdRP genes of SARS-CoV-2.

This study has been authorized by the local Ethical Committee of Emilia Romagna (n. 839/2020).

### Statistical analysis

Median and interquartile range (IQR) were used for all not normally distributed continuous variables and percentages for categorical variables. Nonparametric such as Mann Witney U-test was used to compare continuous variables between unvaccinated and vaccinated groups. Wilcoxon signed-rank test computed the difference between paired samples. The chi-square test and Fisher's exact test were applied to compare categorical variables of baseline characteristics. The Pearson correlation was used to compare RT-PCR and Ct value results. Linear regression analysis was used for determining the relationship between the duration of RT-PCR test positivity and age, gender, ethnicity, dialysis vintage, Kt/V, albumin, diabetes, vaccine status, severe COVID-19, cancer and immunosuppression. Single variables with a $p$ value threshold of < 0.1 in univariate screening were selected for multivariate analysis. A $p$ value < 0.05 was considered statistically significant.

Statistical analyses were performed using SPSS software version 26.0 (IBM, Armonk, NY, USA) and JASP software (University of Amsterdam).

## Results

The diagnosis of COVID-19 was performed 159 times in 151 patients on chronic HD therapy. Eight patients (5.2%) tested positive for SARS-CoV-2 twice. The demographic and clinical characteristics of the enrolled patients are described in Table 1. Median age of these patients was 70.1 (54.3–81.6) years. The overall cohort of patients was characterized by a predominance of males (59.6%) and people of Caucasian origin (63.6%). Median time spent on dialysis before the diagnosis of COVID-19 was 4.2 (1.5–8.5) years. This population included 26.5% of patients with diabetes, 13.4% on immunosuppressants and 3.3% with cancer. As detailed in Fig. 1, the number of cases increased during the spread of Omicron variant in Italy [16, 17]. Overall, median SARS-CoV-2 RT-PCR test positivity lasted 30 (IQR, 21–40) days with a range varying between 5 and 89 days. Duration of RT-PCR positivity was > 20 days in 75.6% of the survivors. Patients with severe illness requiring admission had a longer persistence of positive RT-PCR test (35 [IQR, 28.7–42.2] days) compared to patients with non-severe symptoms (29 [IQR, 20–40.5] days). However, Wilcoxon signed-rank test showed no statistically significant differences between the two groups ($p = 0.16$).

Based on the vaccination status of the patients, the population was subdivided into unvaccinated (n.42; 27.8%) and vaccinated (n.109; 72.1%) groups. Statistical analysis confirmed that unvaccinated patients experienced poor outcome. This subset of the population had a higher rate of severe disease requiring hospitalization (45.2% vs 14.8; $p < 0.001$) and death (40.5% vs 4.6%; $p < 0.001$) compared to the vaccinated patients in our HD population. The median survival days of patients who died due to COVID-19 were 13 (IQR,8–24.2) days. The median survival after COVID-19 in unvaccinated patients was 17 (IQR, 7–26.7) days compared to 9,6 (IQR, 8.5–19) days in vaccinated patients. This difference was not statistically significant ($p = 0.34$).

In unvaccinated survivors, RT-PCR SARS-CoV-2 test positivity lasted 42 (IQR, 31–56) days with a range varying between 11 and 89 days, whereas in vaccinated survivors, RT-PCR test positivity accounted for 27.5 (19.7–35.2) days with a range varying between 5 and 60 days. Statistical analysis revealed a significant difference between the two groups ($p = < 0.001$) (Table 1).

A statistically significant difference in the duration of SARS-CoV-2 RT-PCR test positivity was also noted in patients who experienced reinfection. In these cases, detection of viral RNA on nasopharyngeal mucosa had a significantly shorter persistence (16 [9–43] vs 40 [37–68] days; $p = 0.047$) compared to patients infected once.

Correlation analysis showed that the relationship between RT-PCR and Ct value was positive and

**Table 1** Demographic and clinical characteristics of HD patients with COVID-19

| Variables | All patients (n. 151) | Unvaccinated patients (n. 42) | Vaccinated patients (n. 109) | *p* Value |
|---|---|---|---|---|
| Age, yr | 70.1 (54.3–81.6) | 72.2 (54.8–84.1) | 68.4 (54–80.2) | 0.27 |
| (range) | (20.5–94.3) | (31.7–87.5) | (20.5–94.3) | |
| Male, n. (%) | 90 (59.6) | 27 (64.3) | 63 (57.8) | 0.57 |
| Origin, n. (%) | | | | **0.012** |
| Caucasian | 95 (63.6) | 32 (76.2) | 64 (58.7) | |
| African | 50 (33.1) | 7 (16.7) | 43 (39.4) | |
| Other | 5 (3.3) | 3 (7.1) | 2 (1.8) | |
| Dialysis vintage, yr | 4.2 (1.5–8.5) | 3.9 (1.2–8.8) | 4.3(2.5–7.5) | 0.73 |
| Kt/V | 1.2 (1–1.3) | 1.1 (1–1.3) | 1.2 (1-.4) | 0.61 |
| Hemoglobin, gr/dl | 11.3 (10.4–12.1) | 11.3 (10.5–12.4) | 11.3 (10.2–12.1) | 0.46 |
| Albumin, gr/dl | 3.5 (3.3–3.9) | 3.5 (3.2–4) | 3.5 (3.3–3.9) | 0.6 |
| Phosphorus mg/dL | 5 (4.1–6.3) | 5.1 (4.4–6) | 4.9 (4–6.3) | 0.35 |
| Diabetes, n. (%) | 40 (26.5) | 14 (33.3) | 26 (23.9) | 0.30 |
| Immunosuppressed pts, n. (%) | 20 (13.4) | 5 (12.2) | 15 (13.9) | 0.99 |
| Cancer pts, n. (%) | 5 (3.3) | 1 (2.4) | 4 (3.7) | 0.99 |
| COVID-19 | | | | |
| IgG Ab (BAU/mL) | 7580 (172–17,200) | – | 7580 (172–17,200) | |
| RT-PCR positivity, days* | 30 (21–40) | 42 (31–56) | 27.5 (19.7–35.2) | **< 0.001** |
| Ct value, days* | 18.5 (13.7–26.5) | 16.3 (8.1–31.7) | 18.5 (13.2–27.5) | 0.88 |
| Severe COVID-19, n. (%) | 35 (23.2) | 19 (45.2) | 16 (14.8) | **< 0.001** |
| Death, n. (%) | 22 (14.5) | 17(40.5) | 5 (4.6) | **< 0.001** |

Bold values denote statistical significance at the $p < 0.05$

Ab denotes antibody, RT-PCR reverse transcription polymerase chain reaction

* RT-PCR positivity was calculated only in survivors

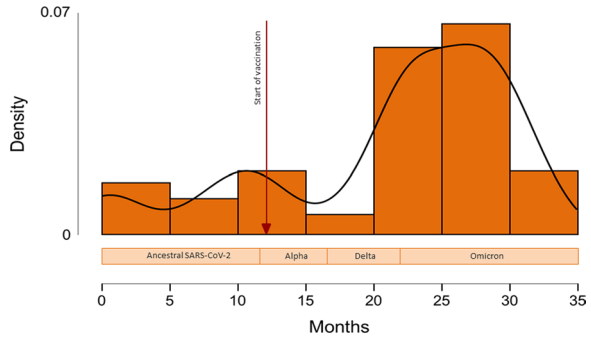


**Fig. 1** Graphic representation of the rate of cases of COVID-19 among patients on chronic HD therapy

statistically significant (r = 0.84; $p = < 0.001$) (Fig. 2). The use of high Ct value, anticipated of 9 (IQR,6–12) days the RT-PCR negative test. Analysis of the duration of viral RNA shedding assessed by RT-PCR and Ct value revealed that the difference between RT-PCR and Ct value (30 [21–40.5] vs 18.5 ([13.7–26.5] days) was statistically significant ($p = < 0.001$).

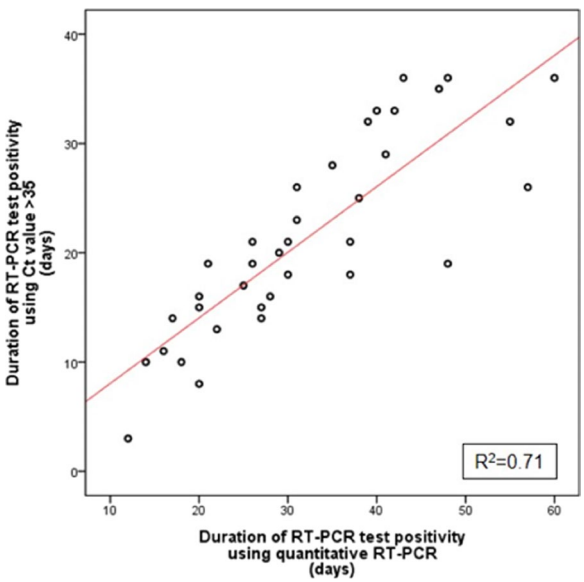


**Fig. 2** Correlation chart displaying the duration of SARS-CoV-2 RT-PCR test positivity assessed by RT-PCR and Ct value in HD patients with COVID-19

**Table 2** Linear regression analysis of predictors of SARS-CoV-2 RT-PCR positivity in survivors

| | Univariate | | | | Multivariate | | | |
|---|---|---|---|---|---|---|---|---|
| | **Beta** | **95%CI** | | ***p* value** | **Beta** | **95%CI** | | ***p* value** |
| Age | 0.27 | 0.09 | 0.41 | **< 0.01** | 0.31 | 0.14 | 0.43 | **< 0.001** |
| Gender (male) | − 0.10 | − 8.25 | 2.38 | 0.28 | | | | |
| Ethnicity (Caucasian) | 0.15 | − 0.91 | 9.66 | 0.10 | − 0.02 | − 5.35 | 4.04 | 0.78 |
| Albumin | − 0.04 | − 9.08 | 6.03 | 0.69 | | | | |
| Kt/V | 0.03 | − 12.24 | 15.80 | 0.80 | | | | |
| Dialysis vintage (year) | − 0.07 | − 0.60 | 0.30 | 0.51 | | | | |
| Diabetes (yes) | 0.04 | − 4.64 | 7.30 | 0.66 | | | | |
| Anti-SARS-CoV-2 Ab | − 0.13 | 0.00 | 0.00 | 0.22 | | | | |
| Immunosuppression (yes) | − 0.03 | − 8.59 | 6.07 | 0.74 | | | | |
| Cancer (yes) | 0.15 | − 1.83 | 27.73 | 0.09 | 0.11 | − 3.81 | 21.6 | 0.16 |
| Anti-COVID-19 vaccination (no) | 0.46 | 10.4 | 21.5 | **< 0.001** | 0.49 | 11.91 | 22.56 | **< 0.001** |
| Severe COVID-19 (yes) | 0.04 | − 6.38 | 9.81 | 0.68 | | | | |

Bold values denote statistical significance at the $p < 0.05$

Multiple linear regression analysis showed that increased age of patients (CI95% 0.1–0.4; $p = < 0.001$) and status of non-vaccination (CI95% 11.9–22.5; $p = < 0.001$) were associated with a longer RT-PCR positivity (Table 2).

At the end of the follow-up, 11 patients died after 170 (IQR, 111–393) days from the resolution of COVID-19. Their median age was 84.1 (68.4–86.3) years and males were 63% of patients. These subjects died after 3.1 (2.3–5.4) years spent on HD therapy. The causes of death were sepsis from bacterial infection (54.5%), cardiovascular events (36.4%) and cancer (9.1%).

## Discussion

The findings of this study showed that patients on chronic HD treatment experienced prolonged RT-PCR test positivity after SARS-CoV-2 infection. The overall duration of RT-PCR positivity was extremely variable as it ranged between a few days to several weeks from COVID-19 diagnosis. In our cohort of 151 HD patients with SARS-CoV-2 infection, detection of viral genome on nasal mucosa lasted for a median of 30 (IQR,21–40) days with a trend toward a longer persistence in patients with severe disease. Surprisingly, in an unvaccinated diabetic patient with mildly symptomatic COVID-19, RT-PCR test for SARS-CoV-2 remained positive for approximately three months. These findings are in line with a previous study conducted on HD patients in Japan, where SARS-CoV-2 RT-PCR positivity lasted 29 ± 6 days from COVID-19 diagnosis [18]. Another study enrolling HD patients with COVID-19 in Belgium showed that the first negative test occurred 34 to 44 days from symptoms [19]. In the peritoneal dialysis population, detection of viral genome on nasopharyngeal mucosa lasted for 26 (IQR, 15–35) days [20]. Evidence shows that immunosuppression or lymphoproliferative cancers are associated with a prolonged SARS-CoV-2 infection [21, 22]. In 10 kidney transplant recipients in Wuhan, RT-PCR test positivity lasted longer than the control group (28.4 ± 9.3 vs 12.2 ± 4.6 days) [23]. An in vitro study showed that viable viruses were detected for more than 3 weeks in nasopharyngeal samples of kidney transplant recipients with COVID-19 [24]. In patients without CKD, detection of SARS-CoV-2 genome by RT-PCR test lasted between 12–20 days after symptom onset [25–28] and slightly longer (median 31 days) in severe cases [29]. A cross-sectional observational study enrolling patients admitted with COVID-19 in two large regional hospitals in Scotland, examined the time to two consecutive negative RT-PCR tests in CKD and non-CKD populations. This study confirmed that the median time from admission to the first negative nasopharyngeal swabs was significantly shorter in patients without CKD (11 [1–71] vs. 18 [1–43] days) ($p = 0.001$). Indeed, GFR was the only variable that was independently associated with the prolonged detection of RT-PCR positivity [30].

Taken together, these data provide evidence that the management of HD patients with COVID-19 is extremely challenging, especially during COVID-19 outbreaks with large surges in infected patients. Indeed, our dialysis staff has been regularly engaged in managing a consistent number of COVID-19 patients requiring prolonged isolation in dedicated rooms to shield uninfected patients from SARS-CoV-2 spread.

Among the findings of this study, we observed that SARS-CoV-2 unvaccinated patients had significantly

longer RT-PCR test positivity (28 vs 42 days) than patients who underwent vaccination. Regression analysis confirmed that anti-SARS-CoV-2 vaccination was independently associated with faster clearance of SARS-CoV-2 genetic material on the nasal mucosa. The vaccine might have had a key role in accelerating the clearance of SARS-CoV-2 and consequently decreasing the time interval between COVID-19 diagnosis and negative test. This latter hypothesis is in line with a previous study documenting the effectiveness of the vaccine in decreasing SARS-CoV-2 transmission by 40% to 50% in the household setting [31]. A similar trend has been noted in the setting of COVID-19 reinfection. Vaccinated patients who experienced a second SARS-CoV-2 infection had a significantly faster resolution than the first infective episode (16 vs 40 days). The natural immunity gained with the resolution of COVID-19 might have been a boost for the immune system in accelerating the clearance of the infection. Older age was another predictor of prolonged clearance in patients on HD. Since aging is associated with a decline in immune function [32], older HD patients may lose the ability to protect against infections [33] and, consequentially, resolution of COVID-19 may be delayed compared to the general population as documented in other studies conducted on CKD patients [33, 34]. In a first report evaluating prolonged shedding of SARS-CoV-2 in the HD population, Otsubo et al [18] corroborated the hypothesis that prolonged persistence of a positive RT-PCR test result may be secondary to the tendency of HD patients with COVID-19 to have impaired antiviral immune response, given their documented susceptibility to severe COVID-19 [35].

Our study also pointed out the results of Ct value, a useful and practical laboratory surrogate of SARS-CoV-2 RNA replication and virus infectivity. Implementation of a Ct cutoff > 35 cycles that defines a low likelihood of SARS-Cov-2 replication, correlated positively with RT-PCR test results. Most importantly, Ct value anticipated a negative RT-PCR test by a median of 9 days, potentially without increasing the risk of SARS-CoV-2 spreading in our dialysis center. Hence, Ct value has the potential to become a new method for monitoring and ending isolation of infected patients with the advantage of reducing the duration of quarantine in HD patients [36], because "positive" RT-PCR test result does not necessarily indicate the presence of a viable virus [18]. Evidence shows that SARS-CoV-2 RT-PCR positivity over 29 days was rarely associated with a viable virus in HD patients [36]. However, there is scarce uniformity about the Ct cutoff value that should be used to predict SARS- CoV-2 growth in virus cultures, as positive viral samples have been identified with PCR Ct values between 17.0 and 39.0 [37–43]. Based on these data, we used a cutoff point over 35 as a conservative measure to avoid the risk of breakthrough infections in our dialysis unit. This cutoff was based on a review of previous studies demonstrating that cultures of viable SARS-CoV-2 are much less likely with higher Ct values [39, 40, 43].

In light of these data, there are no doubts that an alternative test to RT-PCR seems a palatable option to streamline the process of ending isolation and quickly rehabilitate the HD patient to regular daily activities after the resolution of COVID-19. Diagnostic tests, better aligned with SARS-CoV-2 viability, such as Ct value and rapid antigen, are promising options in the diagnostic armamentarium of COVID-19 [44]. However, a cautionary approach is crucial to prevent the spread of the virus among frail HD patients congregating in a high-risk setting [45]. Ct values and cutoffs are not standardized measures and thus cannot be directly comparable between tests. In some complex cases, consultation with an infectious disease specialist is crucial to determining the appropriate management of patients with prolonged viral RNA shedding [12].

The most significant limitations of this study stem from the lack of correlation between the duration of RT-PCR test positivity and COVID-19 variants as well as the absence of data on the viral viability of SARS-CoV-2. The lack of detailed information on the manifestation of COVID-19 is another drawback limiting the inferential analysis of clinical predictors of prolonged RT-PCR positivity. Nevertheless, the findings of this study, extrapolated from a cohort of HD patients regularly screened for SARS-CoV-2 and steadily subjected to preventive measures, improve understanding of COVID-19 pathogenesis in this population and highlight a substantial need for practical recommendations to manage this frail population in case of further outbreaks.

In conclusion, our results confirmed that HD patients with COVID-19 experienced prolonged RT-PCR test positivity which is directly associated with age and inversely associated with anti-SARS-CoV-2 vaccination. Ct value was significantly correlated with RT-PCR value, and its use may reduce the quarantine in HD patients with COVID-19.

**Abbreviations**

| | |
|---|---|
| CDC | Centers for Control and Prevention |
| Ct | Cycle threshold |
| IC | Interval confidence |
| IQR | Interquartile range |
| HD | Hemodialysis |
| OR | Odds ratio |
| RT-PCR | Reverse transcription polymerase chain reaction |

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**Author contributions**
GA: Conceptualization. NM; CF; SG; GL; ST; MP: data curation, writing- original draft preparation. AF; EF; FF: writing- reviewing and editing. NM; CF: investigation. MG; GG; GC; GD; RM: reviewed the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
Most data are reported in the manuscript. Further data are available upon request.

## Declarations

**Ethics approval and consent to participate**
This study has been authorized by the local Ethical Committee of Emilia Romagna (n. 839/2020).

**Consent for publication**
Not applicable.

**Competing interests**
GA received honoraria for events from Baxter Healthcare. The remaining authors declare that they have no competing interests.

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**References**

1. Vacchi C, Meschiari M, Milic J, et al. COVID-19-associated vasculitis and thrombotic complications: from pathological findings to multidisciplinary discussion. Rheumatology (Oxford). 2020;59(12):e147–50. https://doi.org/10.1093/rheumatology/keaa581.
2. Tsuruya K, Fujisaki K. Cerebrovascular disease in end-stage kidney disease. Renal Replacement Therapy. 2016;2(1):29. https://doi.org/10.1186/s41100-016-0044-0.
3. Cianciolo G, Donati G, La Manna G, et al. The cardiovascular burden of end-stage renal disease patients. Minerva Urol Nefrol. 2010;62(1):51–66.
4. Nitta K, Tsuchiya K. Recent advances in the pathophysiology and management of protein-energy wasting in chronic kidney disease. Renal Replacement Therapy. 2016;2(1):4. https://doi.org/10.1186/s41100-016-0015-5.
5. Kikuchi K, Nangaku M, Ryuzaki M, et al. Survival and predictive factors in dialysis patients with COVID-19 in Japan: a nationwide cohort study. Renal Replacement Therapy. 2021;7(1):59. https://doi.org/10.1186/s41100-021-00378-0.
6. Alfano G, Ferrari A, Magistroni R, Fontana F, Cappelli G, Basile C. The frail world of haemodialysis patients in the COVID-19 pandemic era: a systematic scoping review. J Nephrol. 2021;34(5):1387–403. https://doi.org/10.1007/s40620-021-01136-5.
7. Combe C, Kirsch AH, Alfano G, et al. At least 156 reasons to prioritise COVID-19 vaccination in patients receiving in-centre haemodialysis. *Nephrol Dial Transplant*. https://doi.org/10.1093/ndt/gfab007
8. Sugawara Y, Iwagami M, Kikuchi K, et al. Infection prevention measures for patients undergoing hemodialysis during the COVID-19 pandemic in Japan: a nationwide questionnaire survey. Renal Replacement Therapy. 2021;7(1):27. https://doi.org/10.1186/s41100-021-00350-y.
9. Heylen L, Oris E, Wollants E, et al. 128 days of SARS-CoV-2 viral shedding in a haemodialysis patient. Clin Kidney J. 2021;14(4):1284–6. https://doi.org/10.1093/ckj/sfab004.
10. Alfano G, Perrone R, Fontana F, et al. Long-term effects of COVID-19 in a patient on maintenance dialysis. Hemodial Int. 2020;24(4):E50–4. https://doi.org/10.1111/hdi.12859.
11. O'Sullivan ED, Lees JS, Howie KL, et al. Prolonged SARS-CoV-2 viral shedding in patients with chronic kidney disease. Nephrology (Carlton). 2021;26(4):328–32. https://doi.org/10.1111/nep.13844.
12. CDC. Recommendation for Ending Isolation. Centers for Disease Control and Prevention. Published February 11, 2020. Accessed January 15, 2023. https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html
13. Guidance-for-discharge-and-ending-of-isolation-of-people-with-COVID-19-third-update.pdf. Accessed June 3, 2022. https://www.ecdc.europa.eu/sites/default/files/documents/Guidance-for-discharge-and-ending-of-isolation-of-people-with-COVID-19-third-update.pdf
14. Basile C, Combe C, Pizzarelli F, et al. Recommendations for the prevention, mitigation and containment of the emerging SARS-CoV-2 (COVID-19) pandemic in haemodialysis centres. Nephrol Dial Transplant. 2020;35(5):737–41. https://doi.org/10.1093/ndt/gfaa069.
15. Platten M, Hoffmann D, Grosser R, et al. SARS-CoV-2, CT-Values, and infectivity—conclusions to be drawn from side observations. Viruses. 2021;13(8):1459. https://doi.org/10.3390/v13081459.
16. Marziano V, Guzzetta G, Menegale F, et al. The decline of COVID-19 severity and lethality over two years of pandemic.
17. La Rosa G, Iaconelli M, Veneri C, et al. The rapid spread of SARS-COV-2 Omicron variant in Italy reflected early through wastewater surveillance. Sci Total Environ. 2022;837:155767. https://doi.org/10.1016/j.scitotenv.2022.155767.
18. Otsubo S, Aoyama Y, Kinoshita K, et al. Prolonged shedding of SARS-CoV-2 in COVID-19 infected hemodialysis patients. Ther Apher Dial. 2021;25(3):356–8. https://doi.org/10.1111/1744-9987.13566.
19. De Vriese AS, Reynders M. IgG antibody response to SARS-CoV-2 infection and viral RNA persistence in patients on maintenance hemodialysis. Am J Kidney Dis. 2020;76(3):440–1. https://doi.org/10.1053/j.ajkd.2020.05.009.
20. Alfano G, Fontana F, Giovanella S, et al. Prevalence, clinical course and outcomes of COVID-19 in peritoneal dialysis (PD) patients: a single-center experience. Clin Exp Nephrol. 2023;27(2):171–8. https://doi.org/10.1007/s10157-022-02283-0.
21. Choi B, Choudhary MC, Regan J, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med. 2020;383(23):2291–3. https://doi.org/10.1056/NEJMc2031364.
22. Cesaro S, Ljungman P, Mikulska M, et al. Recommendations for the management of COVID-19 in patients with haematological malignancies or haematopoietic cell transplantation, from the 2021 European Conference on Infections in Leukaemia (ECIL 9). *Leukemia*. 2022; 36(6):1467–1480. https://doi.org/10.1038/s41375-022-01578-1
23. Zhu L, Gong N, Liu B, et al. Coronavirus disease 2019 pneumonia in immunosuppressed renal transplant recipients: a summary of 10 confirmed cases in Wuhan. China Eur Urol. 2020;77(6):748–54. https://doi.org/10.1016/j.eururo.2020.03.039.
24. Benotmane I, Risch S, Doderer-Lang C, Caillard S, Fafi-Kremer S. Long-term shedding of viable SARS-CoV-2 in kidney transplant recipients with COVID-19. Am J Transplant. 2021;21(8):2871–5. https://doi.org/10.1111/ajt.16636.
25. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020;395(10229):1054–62. https://doi.org/10.1016/S0140-6736(20)30566-3.
26. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. JAMA. 2020;323(15):1488–94. https://doi.org/10.1001/jama.2020.3204.

27. Zheng S, Fan J, Yu F, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ. 2020;369:m1443. https://doi.org/10.1136/bmj.m1443.
28. Zhou Y, Ding F, Bao W, et al. Clinical features in coronavirus disease 2019 (COVID-19) patients with early clearance and prolonged shedding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. Ann Transl Med. 2021;9(8):665. https://doi.org/10.21037/atm-21-445.
29. Zhou B, She J, Wang Y, Ma X. Duration of viral shedding of discharged patients with severe COVID-19. Clin Infect Dis. 2020;71(16):2240–2. https://doi.org/10.1093/cid/ciaa451.
30. O'Sullivan ED, Lees JS, Howie KL, et al. Prolonged SARS-CoV -2 viral shedding in patients with chronic kidney disease. Nephrology. 2021;26(4):328–32. https://doi.org/10.1111/nep.13844.
31. Harris RJ, Hall JA, Zaidi A, Andrews NJ, Dunbar JK, Dabrera G. Effect of vaccination on household transmission of SARS-CoV-2 in England. *N Engl J Med*. 2021:NEJMc2107717. https://doi.org/10.1056/NEJMc2107717
32. Sadighi Akha AA. Aging and the immune system: an overview. J Immunol Methods. 2018;463:21–6. https://doi.org/10.1016/j.jim.2018.08.005.
33. Ducloux D, Legendre M, Bamoulid J, Saas P, Courivaud C, Crepin T. End-stage renal disease-related accelerated immune senescence: is rejuvenation of the immune system a therapeutic goal? Front Med (Lausanne). 2021;8:720402. https://doi.org/10.3389/fmed.2021.720402.
34. Hilbrands LB, Duivenvoorden R, Vart P, et al. COVID-19-related mortality in kidney transplant and dialysis patients: results of the ERACODA collaboration. Nephrol Dial Transplant. 2020;35(11):1973–83. https://doi.org/10.1093/ndt/gfaa261.
35. Alfano G, Fontana F, Ferrari A, et al. Which criteria should we use to end isolation in hemodialysis patients with COVID-19? *Clinical Kidney Journal*. Published online April 30, 2022:sfac115. doi:https://doi.org/10.1093/ckj/sfac115
36. Lacson E, Weiner D, Majchrzak K, et al. Prolonged live SARS-CoV-2 shedding in a maintenance dialysis patient. Kidney Med. 2020;3(2):309–11. https://doi.org/10.1016/j.xkme.2020.12.001.
37. Romero-Gómez MP, Gómez-Sebastian S, Cendejas-Bueno E, Montero-Vega MD, Mingorance J, García-Rodríguez J. Ct value is not enough to discriminate patients harbouring infective virus. J Infect. 2021;82(3):e35–7. https://doi.org/10.1016/j.jinf.2020.11.025.
38. Kujawski SA, Wong KK, Collins JP, et al. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. Nat Med. 2020;26(6):861–8. https://doi.org/10.1038/s41591-020-0877-5.
39. Basile K, McPhie K, Carter I, et al. Cell-based culture informs infectivity and safe de-isolation assessments in patients with coronavirus disease 2019. Clin Infect Dis. 2021;73(9):e2952–9. https://doi.org/10.1093/cid/ciaa1579.
40. Gniazdowski V, Paul Morris C, Wohl S, et al. Repeated coronavirus disease 2019 molecular testing: correlation of severe acute respiratory syndrome coronavirus 2 culture with molecular assays and cycle thresholds. Clin Infect Dis. 2021;73(4):e860–9. https://doi.org/10.1093/cid/ciaa1616.
41. Bullard J, Dust K, Funk D, et al. Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. Clin Infect Dis. 2020;71(10):2663–6. https://doi.org/10.1093/cid/ciaa638.
42. Arons MM, Hatfield KM, Reddy SC, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med. 2020;382(22):2081–90. https://doi.org/10.1056/NEJMoa2008457.
43. La Scola B, Le Bideau M, Andreani J, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis. 2020;39(6):1059–61. https://doi.org/10.1007/s10096-020-03913-9.
44. Ferrari A, Alfano G, Guaraldi G. Persistent SARS-CoV-2 positivity: an intriguing puzzle among reinfection, RNA remnants and genomic integration in COVID-19. Infect Dis Clin Pract (Baltim Md). 2021;29(5):e328–9. https://doi.org/10.1097/IPC.0000000000001043.
45. Alfano G, Fontana F, Morisi N, et al. Ethical challenges in managing unvaccinated patients receiving chronic in-centre haemodialysis. Clin Kidney J. 2022;15(4):615–7. https://doi.org/10.1093/ckj/sfab276.


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