Infection Control & Hospital Epidemiology (2023), **44**, 659–662
doi:10.1017/ice.2021.502

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Concise Communication

Persistence of infectivity in elderly individuals diagnosed with severe acute respiratory coronavirus virus 2 (SARS-CoV-2) infection 10 days after onset of symptoms: A cross-sectional study

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Abstract

We performed viral culture of nasopharyngeal specimens in individuals aged 79 and older, infected with severe acute respiratory coronavirus virus 2 (SARS-CoV-2), 10 days after symptom onset. A positive viral culture was obtained in 10 (45%) of 22 participants, including 4 (33%) of 12 individuals with improving symptoms. The results of this small study suggest that infectivity may be prolonged among older individuals.

(Received 28 July 2021; accepted 22 November 2021; electronically published 6 December 2021)

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Infected individuals must be placed under isolation precautions until infectivity is resolved.[1] The current recommendation is based on studies that used viral culture as an indicator of infectivity and requires isolation for 10 days for nonsevere infections in immunocompetent individuals, provided that symptoms are improving.[1,2] However, these studies enrolled relatively young individuals and may not be generalizable to older patients.[1,3,4] Furthermore, some studies have reported a longer duration of SARS-CoV-2 RNA shedding by reverse transcription polymerase chain reaction (RT-PCR) among older patients.[5] We assessed the presence of infectious SARS-CoV-2 virus in elderly individuals with COVID-19, 10 days after symptom onset, using viral culture as a surrogate for infectivity.

Methods

Population and setting

In this prospective cross-sectional study, we included a convenience sample of unvaccinated individuals aged >79 years with COVID-19 confirmed by RT-PCR from 3 long-term care facilities and 1 acute-care institution in Montreal, Canada, between November 8, 2020, and January 25, 2021. Participants were identified using institutional infection control databases. Following verbal consent, a nasopharyngeal sample was collected on day 10 after symptom onset using a flocked swab (FLOQSwabs, Copan Italia, Brescia, Italy) and placed in 3 mL universal viral transport media (Copan Italia). The samples were kept at −80°C until RT-PCR and viral culture. Clinical data (ie, immunosupression, date of symptom onset, symptomatology, clinical outcomes) and the cycle threshold (Ct) value of the first positive RT-PCR were extracted from patient charts.

Laboratory methods

Viral cultures were performed on Vero E6 cells in 4-mL shell vials as previously described using a 0.1-mL aliquot of specimen as inoculum.[6] Cultures were kept for 15 days and monitored for a cytopathic effect (CPE) every other day.

On initial (day 0) samples, viral RNA was extracted using the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Thermo Scientific, Waltham MA). RT-PCR was performed on a LightCycler LC480 (Roche) using TaqPath RT-qPCR Master Mix (Thermo Scientific) and primers and probe targeting the E gene as described previously.[7] Samples collected on day 10 and culture supernatants were processed with an in-house RT-PCR targeting the N gene as previously described.[6] We used the following forward, reverse and probe sequences: AACCAGAATGGAGAACGCAGTG, CGGTGAACCAAGACGCAGTATTAT and CGATCAAAACAA CGTCGGCCCCAAGGTTTAC.[6]

Statistical analyses

The primary outcome was culture positivity, defined as the detection of a CPE combined with a RT-PCR Ct value on the supernatant that is lower than the Ct value of the original specimen. To investigate factors associated with culture positivity, the Fisher

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Cite this article: Longtin Y, *et al.* (2023). Persistence of infectivity in elderly individuals diagnosed with severe acute respiratory coronavirus virus 2 (SARS-CoV-2) infection 10 days after onset of symptoms: A cross-sectional study. *Infection Control & Hospital Epidemiology*, 44: 659–662, https://doi.org/10.1017/ice.2021.502

Table 1. Comparison of Patients With Positive or Negative Culture for SARS-CoV-2 on Day 10 of Infection

Characteristic	Total (N=22), No. (%)	Culture Positive (N=10), No. (%)	Culture Negative (N=12), No. (%)	*P* Value
Sex, female	12 (55)	5 (50)	7 (58)	1.00
Age, median y (IQR)	90 (86–96)	90 (86–98)	90 (84–96)	.69
Immunosuppression	4 (18)	2 (20)	2 (17)	1.00
Reception of steroids	13 (59)	8 (80)	5 (42)	.10
Outcome				
Hospitalized	8 (36)	5 (50)	3 (25)	.38
Death	7 (32)	3 (30)	4 (33)	1.00
Duration of isolation precautions, d (IQR)	13 (11–16)	14 (11–22)	12 (11–16)	.38
Symptoms[1]				
Cough	16 (73)	8 (80)	8 (67)	.65
Fever	11 (50)	6 (60)	5 (42)	.67
Respiratory distress	7 (32)	4 (40)	3 (25)	.65
Other COVID-19–compatible symptoms[2]	16 (73)	7 (70)	9 (75)	1.00
Absence of risk factors for prolonged shedding[3]	4 (18)	2 (20)	2 (17)	1.00
Symptomatology on day 10 of infection				
Resolved symptoms	8 (36)	3 (30)	5 (42)	.68
Persistent but improving symptoms	4 (18)	1 (10)	3 (25)	.59
Persistent and not improving symptoms	10 (45)	6 (60)	4 (33)	.39
Persistent fever[4]	4 (18)	2 (20)	2 (17)	1.00
Laboratory				
Ct value on RT-PCR on day of diagnosis[5] (IQR)	21 (16–27)	24 (15–27)	21 (18–29)	.73
Ct value on RT-PCR on day 10 of infection (IQR)[6]	21 (16–24)	17 (15–19)	23 (21–26)	.002

Note. Ct, cycle threshold; RT-PCR, reverse-transcription polymerase chain reaction; IQR, interquartile range.
[1]Symptoms reported at least once during SARS-CoV-2 infection.
[2]Includes lethargy (n=8), decreased appetite (n=6), weakness (n=3), diarrhea (n=3), nausea (n=1), confusion (n=1), hyperglycemia (n=1), hoarse voice (n=1), nasal congestion (n=1), wheezing (n=1), and syncope (n=1).
[3]Defined as all of the following: resolution of fever by day 10, symptoms improving or completely resolved on day 10, absence of immunosuppression, absence of steroid use, absence of hospitalization.
[4]Defined as fever on the day of testing or the preceding day.
[5]n=17 (2 missing values).
[6]2 samples had no SARS-CoV-2 detectable by RT-PCR and were excluded.

exact test and the Mann-Whitney U test were performed as appropriate. To investigate the capacity of Ct value on day 10 to predict culture positivity, a receiver operating characteristics (ROC) curve was plotted to determine the probability of a true positive result as a function of the probability of a false positive result for all possible Ct values. A $P < .05$ was considered significant. The study was approved by the local research ethics committees.

Results

Overall, 22 participants were recruited. The median age was 90 years old (interquartile range [IQR], 86–96) and 12 (55%) were female (Table 1). Also, 4 patients (18%) were immunosuppressed, 13 (59%) received steroids to treat their infection, 8 (36%) were hospitalized, and 7 (32%) died. The initial sample to confirm the diagnosis was taken within 1 day of symptom onset in 21 (95%) of 22 and had a median RT-PCR Ct value of 21 (IQR, 16–27).

By day 10 after symptom onset, 8 (36%) of 22 had no residual symptom, 4 (18%) of 22 were still symptomatic but improving, whereas 10 (45%) of 22 had no symptom improvement. Specimens on day 10 had a median Ct of 21 (IQR, 16–24). A positive viral culture was obtained in 10 (45%) of 22 samples. Detection of a CPE occurred early; 6 (60%) of 10 samples demonstrated a CPE by incubation day 3, and 9 (90%) of 10 by incubation day 8.

A positive culture was obtained for 6 (60%) of 10 individuals whose symptoms were not improving on day 10 and for 4 (33%) of 12 of those with improving or resolved symptoms. Individuals with positive cultures tended to be more frequently hospitalized than culture-negative individuals: 5 (50%) of 10 versus 3 (25%) of 12, respectively. They were also more likely to have received steroids than those with negative cultures: 8 (80%) of 10 versus 5 (42%) of 12. However, individuals with positive cultures were less likely to die than those with negative cultures: 3 (30%) of 10 versus 4 (33%) of 12, respectively. Notably, these differences were not statistically significant. Among patients without a recognized risk factor for prolonged shedding, half (2 of 4) had a positive culture on day 10.

The Ct value of the initial sample did not predict culture positivity (median Ct of participants with and without a positive

https://doi.org/10.1017/ice.2021.502 Published online by Cambridge University Press

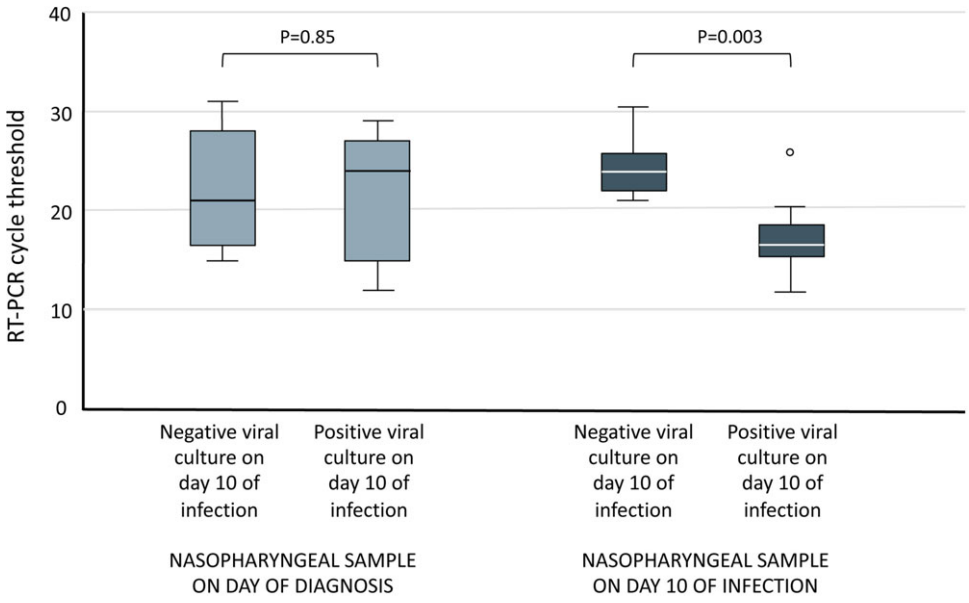

Fig. 1. Box plot showing the cycle threshold value of SARS-CoV-2 RT-PCR of nasopharyngeal swabs taken on the day of diagnosis and on day 10 after onset of symptoms from elderly patients infected with COVID-19, stratified by persistence of infectivity on day 10 after onset of symptoms. The horizontal line in each box indicates the median, whereas the top and bottom lines represent the 75th and 25th percentiles, respectively. Error bars represent 95% confidence intervals, and the dot represents an outlier.
Note. RT-PCR, detection of SARS-CoV-2 by reverse-transcription polymerase chain reaction.

culture on day 10, 24 vs 21; $P = .73$) (Fig. 1). In contrast, the Ct value of the sample collected on day 10 predicted culture positivity (median Ct of positive vs negative cultures, 17 (IQR, 15–19) vs 23 (IQR, 21–26), respectively; $P = .003$). The performance of the day-10 Ct value in predicting culture positivity is shown through the receiver operating characteristics (ROC) curve (Supplementary Fig. 1 online). The area under the ROC curve was 0.90 (95% CI, 0.74–1.00). Using a cutoff Ct of 20.7 had 90% sensitivity (9 of 10 patients) and 90% specificity (9 of 10 patients) to predict culture positivity.

Discussion

The belief that patients with COVID-19 remain infectious for <10 days stems from studies conducted among younger populations, with an average or median age ranging from 33 to 57.[3,4] This notion may not be generalizable to patients over the age of 79; ~50% of individuals in our study were still shedding infectious viral particles 10 days after symptom onset. Furthermore, clinical improvement was not predictive of loss of infectivity; ~33% those with resolved or improving symptoms remained infective. In contrast, a lower cycle threshold (indicating a greater quantity of viral RNA in the sample) was a predictor of culture positivity on day 10 of COVID-19, as reported elsewhere.[2,3,8]

Few studies have investigated infectivity specifically among older individuals. In British long-term care homes, infectious virus could be recovered for up to 13 days among residents (median age, 85) compared to up to 7 days among staff (median age, 47).[9] Among nursing home residents in Washington, 4 of 8 samples collected between days 7 and 13 after symptom onset were positive by viral culture.[2] By contrast, recovery of replication-competent SARS-CoV-2 was 8 days or less after diagnosis in 8 of 9 nursing home residents in Arkansas, although infectivity persisted for 19 days in 1 immunocompromised individual.[10]

Our study has several limitations. The small sample size limits its generalizability. We did not collect data on comorbidities and other predictors of prolonged infectivity. Cultures were performed after a freeze–thaw cycle that may affect the integrity of the virus. We did not perform epidemiological investigations to correlate culture positivity and transmissibility to other individuals. Most individuals had risk factors for prolonged viral shedding. Still, persistent infectivity in our elderly population was much more frequent than previously reported among severe cases in younger individuals in which ~5% remain infectious by day 10.[8]

In conclusion, this investigation suggests that infectivity may be longer than recognized among older individuals and appear to be predicted by low Ct values on day 10 of infection. Further studies are required to determine whether isolation precaution recommendations should be modified among the older individuals.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/ice.2021.502

Acknowledgments. We thank the microbiology technicians of the virology laboratory at the Laboratoire de Santé Publique du Québec for their assistance in conducting the viral cultures.

Financial support. Laboratoire de Santé Publique du Québec. Dr Yves Longtin's research is supported by the Fonds de Recherche du Québec en Santé.

Conflicts of interest. Yves Longtin declares research grants outside of the submitted work from Merck, Gojo and Becton Dickinson. All other authors declare no competing interests.

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https://doi.org/10.1017/ice.2021.502 Published online by Cambridge University Press