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Short Communication

Initial SARS-CoV-2 PCR crossing point does not predict hospitalization and duration of PCR positivity

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Abstract This study aimed to determine if the crossing point of the initial positive SARS-CoV-2 PCR test correlated with patient demographics, subsequent hospitalization, or duration of positivity. Seventy-three patients with two or more positive PCR tests had a median time of 23 days to two consecutive negative results.

Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory tract infection caused by a single-stranded RNA virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Molecular testing methods, primarily reverse transcription polymerase chain reaction (RT-PCR), have been the diagnostic test-of-choice for identifying those with a current infection. Previous studies have shown that the PCR crossing point (Cp) (i.e., cycle threshold) values correlate inversely with the viral load. Some have proposed the use of the Cp as a surrogate measure of disease severity and viral shedding.[1,2] Another study monitored viral load over time (as determined by PCR Cp values), and showed COVID-19 patients to have a lower PCR Cp value (i.e., higher concentration of viral RNA) shortly after symptom onset,

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implicating higher viral loads early in infection.[3] This is consistent with published data demonstrating peak concentrations of the virus in upper respiratory specimens before day 5 following symptom onset.[4]

While published data suggest that SARS-CoV-2 is most reliably detected in upper respiratory specimens early in the disease course, SARS-CoV-2 has been detected for greater than 20 days in upper and lower respiratory samples, and in some cases, following the resolution of clinical symptoms and development of antibodies.[3,4] One proposed clinical use for PCR Cp values is in predicting disease severity and the likelihood of a persistent positive RT-PCR test result in patients diagnosed with COVID-19. This issue of SARS-CoV-2 RNA persistence is of importance to infection prevention and control strategies, as many COVID-19 patients are undergoing routine re-testing to inform decisions regarding the discontinuation of quarantine and allow for return to work. In this study, we assessed the potential correlation of initial PCR Cp values with duration of PCR positivity and disease severity.

Methods

Study design

This study was reviewed and approved by the institutional review board at our center. No samples were collected for the sole use of this study, as the data were reviewed following routine SARS-CoV-2 testing. At the time of data collection, all patients with research authorization and more than one positive result by an emergency use authorized laboratory-developed SARS-CoV-2 PCR assay were included in this analysis.[5] PCR crossing point information was obtained from the laboratory information system and cross-referenced with basic patient demographics.

SARS-CoV-2 real time RT-PCR assay

The laboratory developed test method, including primer/probe sequences and thermocycling conditions, are described in detail by Rodino et al.[5] In brief, respiratory specimens were added to NucliSENS® lysis buffer (bioMérieux; Durham, NC) in a class II biosafety cabinet. After lysis, nucleic acid extraction was performed on the eMAG® or easyMAG® platform (bioMérieux; Durham, NC) and purified nucleic acid tested for the presence of two target regions within the SARS-CoV-2 genome utilizing TaqMan™ real-time RT-PCR technology on the LightCycler 480 (Roche Molecular, Indianapolis, IN). The assay targets the SARS-CoV-2 nucleocapsid (NUC) and open reading frame (ORF) genes. In addition, each reaction includes an internal control (i.e., murine hepatitis virus sequence) that must be detected in negative samples or the reaction is invalid. Following an initial reverse transcription step, fluorescence output is monitored for a total of 45 cycles. Due to differences in target specificity, the detection of the NUC target (alone or in combination with ORF) is considered positive for SARS-CoV-2, while detection of ORF alone is considered indeterminate. This test has been granted Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration (FDA).

Statistical analysis

Analyses included descriptive statistics of the cohort, generation of a Kaplan–Meier curve, and development of logistic and linear regression models predicting hospital admission and repeat PCR Cps, respectively. Statistical analysis was performed using JMP®, Version 14 (SAS Institute Inc., Cary, NC, 1989–2019). For the purpose of this study, two consecutive negative results are defined as two negative PCR results on samples collected at least 24 h apart. The time to two consecutive negative results is measured on the date of the first negative PCR result.

Results

Patient demographics

Over a 6 week time period spanning March to April 2020, 73 patients met inclusion criteria with a total of 187 positive tests performed on these patients. The median age was 50 (IQR 32–61) and 62% were female (Table 1). The median NUC and ORF Cps of the initial tests were 24.67 and 24.88, respectively. The median NUC and ORF Cps of the last positive PCR tests were 32.7 and 31.9, respectively. At the time of this analysis, 56 (77%) out of 73 patients had tested negative on at least 2 separate specimen collections.

Table 1 Cohort demographics.

Age Range	0–65	>65	Total
No.	59	14	73
No. (%) male	21 (36%)	7 (50%)	28 (38%)
No. (%) hospitalized	7 (12%)	6 (43%)	13 (17.8%)
No. (%) with Two consecutive negative results	47 (80%)	9 (64%)	56 (77%)
Initial NUC Cp (IQR)	24.9 (20.8–29.1)	24.0 (20.9–25.7)	24.5 (20.8–28.7)
Initial ORF Cp (IQR)	25.4 (21.9–27.9)	23.6 (21.6–25.7)	24.9 (21.7–27.2)

No., number; Cp, crossing point; IQR, interquartile range.

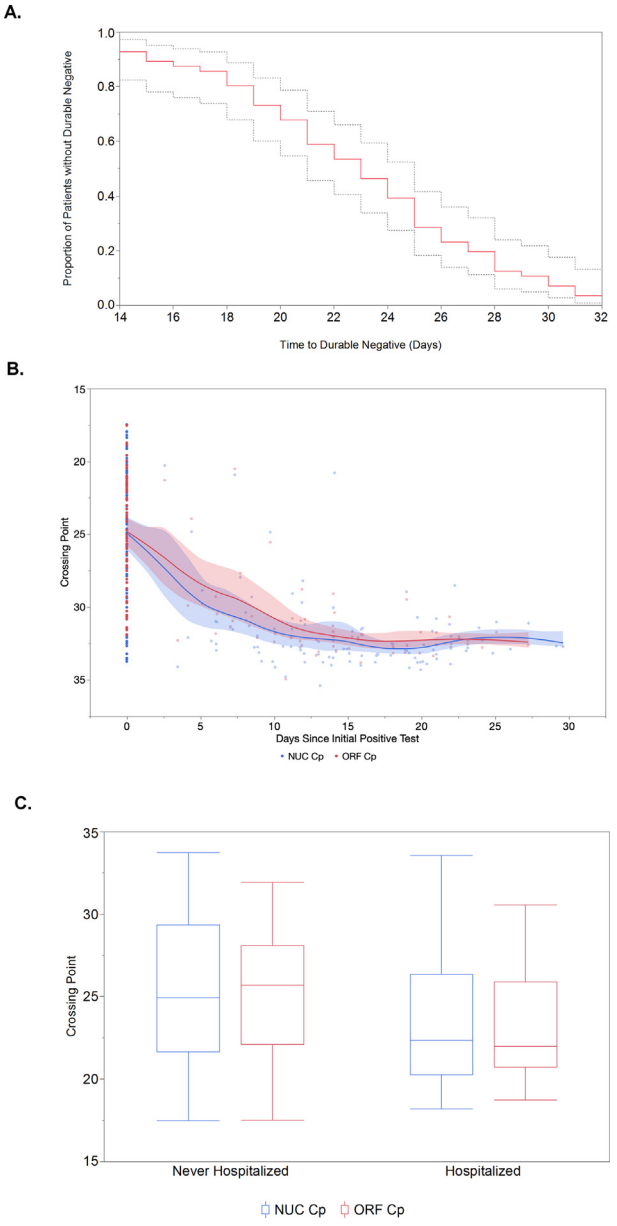

Fig. 1. Duration of time to two consecutive negative results is not related to initial crossing points or hospitalization status. A. Kaplan—Meier curve shows the duration of time to two consecutive negative results (defined as two consecutive negative tests at least 24 h apart). The median time to two consecutive negative results was 23 days. Confidence interval (95%) represented by dotted line. B. Linear regression modeling shows Cps over time from the patients' initial positive test. Each point represents a Cp for a given patient at a given time after the individual's first positive test; red points denote ORF Cp, while blue points denote NUC Cp. C. Boxplot shows the median crossing point of the initial test for the patient cohort based on hospitalization status. The median NUC Cp (blue boxes) for the never hospitalized group was 24.9 (IQR 21.7—29.3) and hospitalized group was 22.4 (IQR 20.3—26.4). The median ORF Cp (red boxes) for the never hospitalized group was 25.7 (22.1—28.1) and hospitalized group was 21.9 (20.7—25.9).

Time to two consecutive negative results for SARS-CoV-2 is not strongly related to initial crossing points

The Kaplan—Meier curve shows the time to two consecutive negative results across the entire cohort (Fig. 1A). This cohort's median time to two consecutive negative results was 23 days (range: 14—32 days). Of the 73 patients with multiple tests, 44 had two tests, 19 had three, 8 had four, and 2 had five that were positive (median 2 tests, IQR 2—3). In those patients who ultimately had two consecutive negative results, 31 required two tests, 17 required three, 7 required four, and 1 required five tests prior to achieving two consecutive negative results (median 2 tests, IQR 2—3). There was no relationship between the initial Cp of the ORF target and the number of days to a two consecutive negative results. However, for every single unit increase in the initial Cp of the NUC target, the time to two consecutive negative results decreased by 0.30 days (95% CI 0.02—0.57). This small effect was similar after adjusting for patient age and sex. As expected, Cp values showed a trend of increasing over time (Fig. 1B). In single variable linear regression, neither age nor sex was significant predictors of initial Cp for either target.

Initial PCR crossing points of NUC and ORF do not predict current hospitalization status, may predict future hospitalization

There was no relationship between the initial Cps for both NUC and ORF and hospitalization status (Fig. 1C). In single variable logistic regression, the first test Cp for the NUC target did not increase odds of subsequent hospital admission (Odds Ratio [OR] 0.90 [95% 0.79—1.01]). The first test Cp for the ORF target was mildly predictive of subsequent hospitalization (OR 0.84 [95% 0.70—0.98]). Location of sample collection (i.e., inpatient vs outpatient) could not be reliably predicted by the Cps for either NUC (OR 1.01, 0.92—1.09) or ORF (OR 0.99, 0.89—1.11) suggesting that Cp may not be a marker of current disease severity.

Discussion

In this study cohort that included both outpatient and hospitalized individuals, the PCR Cp value was not a strong predictor of the time to a negative result or current disease severity. There was evidence that the initial ORF Cp may be mildly predictive of subsequent hospitalization (OR 0.84 [95% 0.70—0.98]). These data suggest that the disease severity and the duration to PCR negativity are not dependent upon the initial PCR Cp value. Additionally, there was no difference in initial Cps for age and gender, which is consistent with previously published findings that age and gender did not impact the viral load or duration of viral shedding.[6,7]

This study has several limitations, including the inability to perform a chart review for most patients, as many samples were submitted through our reference laboratory

operation. Although the median NUC and ORF Cp values were lower (i.e., more strongly positive) in the hospitalized versus non-hospitalized patients, the differences did not reach statistical significance. Further studies assessing this correlation using a larger patient cohort may be needed. Also, we did not evaluate the time from symptom onset to sample collection. However, there was still a trend of increasing Cp with time. Additionally, not all patients had the same duration of follow up, with 17 of the 73 patients still testing positive at the completion of this study. Another limitation of this study is that we used a single real-time PCR method, so Cp results cannot be generalized to other molecular assays for SARS-CoV-2. Finally, our results do not provide any conclusions on the period of viral infectiousness, which can only be assessed through culture studies and contact tracing of positive cases. The prolonged PCR positivity observed in our study is comparable to previous reports showing that PCR positivity may last up to 48 days.[6,8] Furthermore, temporal fluctuations in viral loads have been reported in the literature, with reports of RT-PCR positivity after clinical resolution and several days after two negative RT-PCR tests done after 2 weeks of symptom onset.[9,10]

Prolonged detection of viral RNA has significant implications on current isolation/quarantine and work restriction policies. At the time of this study, the recommendation of the Centers for Disease Control and Prevention (CDC) was to follow a test-based strategy after symptom resolution, requiring two consecutive negative molecular tests from nasopharyngeal swabs collected at least 24 h apart. Our findings suggested that reevaluation of current criteria for return to work and ending home isolation/quarantine may be needed and support the updated CDC guidelines. The CDC guidelines now do not recommend after a test-based strategy for ending quarantine.[11]

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Declaration of competing interest

The authors have no relevant conflicts of interest to disclose related to the work in this study.

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References

1. Fuller JA, Njenga MK, Bigogo G, Aura B, Ope MO, Nderitu L, et al. Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity, Kenya. *J Med Virol* 2013;**85**(5):924—32.
2. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008;**3**(6):1101—8.
3. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; **395**(10229):1054—62.
4. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020 May;**581**(7809):465—9. https://doi.org/10.1038/s41586-020-2196-x. Epub 2020 Apr 1. PMID: 32235945.
5. Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Germer JJ, Fernholz E, et al. Evaluation of saline, phosphate-buffered saline, and minimum essential medium as potential alternatives to viral transport media for SARS-CoV-2 testing. *J Clin Microbiol* 2020 May 26;**58**(6):e00590-20. https://doi.org/10.1128/JCM.00590-20. PMID: 32229604; PMCID: PMC7269412.
6. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* 2020;**26**(5):672—5.
7. Hung IF, Cheng VC, Wu AK, Tang BS, Chan KH, Chu CM, et al. Viral loads in clinical specimens and SARS manifestations. *Emerg Infect Dis* 2004;**10**(9):1550—7.
8. Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, Behillil S, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Infect Dis* 2020; **20**(6):697—706.
9. Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. PCR assays turned positive in 25 discharged COVID-19 patients. *Clin Infect Dis* 2020 Apr 8:ciaa398. https://doi.org/10.1093/cid/ciaa398.
10. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR test results in patients recovered from COVID-19. *JAMA* 2020 Apr 21; **323**(15):1502—3. https://doi.org/10.1001/jama.2020.2783. PMID: 32105304; PMCID: PMC7047852.
11. Prevention CfDCa. *Coronavirus Disease 2019 (COVID019) Discontinuing Transmission-Based Precautions*. 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-hospitalized-patients.html.