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SHORT COMMUNICATION

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Cycle threshold values and SARS-CoV-2: Relationship to demographic characteristics and disease severity

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Abstract

Cycle threshold (Ct), or the number of cycles required to amplify viral RNA to a detectable level, provides an estimate of viral load. Previous studies have demonstrated mixed results in regard to the association between SARS-CoV-2 Ct from real-time reverse transcriptase PCR (rRT-PCR) testing to patient outcomes, and there is less data on the relationship between Ct and patient characteristics. This was a retrospective study of 256 patients tested at a tertiary care emergency department from March to July 2020 via nasopharyngeal rRT-PCR testing utilizing the Abbott M2000 SARS-CoV-2 assay. Kruskal–Wallis, univariable, and multivariable logistic regression were used where appropriate for analysis. There were no significant differences in Ct value by demographic characteristics including age, sex, race, or ethnicity. Ct increased with time since symptom onset ($p < 0.001$), and increasing Ct was associated with increased odds of severe disease (odds ratio: 1.05, 95% confidence interval: 1.0–1.11). Ct was not found to be associated with patient demographic characteristics and increasing Ct was found to be associated with increased odds of severe disease. Continued study will allow us to better comprehend the complex factors that contribute to the risk for severe outcomes due to SARS-CoV-2 infection.

1 | INTRODUCTION

Since its discovery in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has been responsible for more than 180 million cases and almost 4 million deaths worldwide.[1] Despite substantial advances in our knowledge of this disease, research continues to fully understand SARS-CoV-2, a clade within the family *Coronaviridae*, genus *Betacoronavirus*, subgenus *Sarbecovirus*, species *Severe acute respiratory syndrome-related virus*,[2] particularly in understanding factors related to patient prognosis after infection. Early studies have found that certain individuals are at greater risk for poor outcomes after developing COVID-19, including older individuals, those who are obese, and patients with multiple comorbid conditions.[3–5]

The primary method of diagnosis of SARS-CoV-2 is currently by real-time reverse transcriptase PCR (rRT-PCR). These rRT-PCR tests amplify and detect viral genetic sequences, and a fluorescence signal increases proportionally to the amount of amplified nucleic acid, enabling the quantification of RNA in the sample.[6] This signal is expressed as the cycle threshold (Ct), or the number of cycles required to amplify viral RNA to a detectable level, which provides a quantifiable estimate of viral load.[6] The Ct value is inversely related to viral load[6] and has been used as a proxy for viral in a number of studies[7–10] given the more resource intensive effort required of direct viral load measurement.

Previous studies have found mixed results with regard to the association between Ct and patient outcomes, including risk for intubation, admission to an intensive care unit, and in-hospital

Jessica Penney and Amanda Jung contributed equally to this study.

survival.[7,8,11] The purpose of this observational study was to evaluate the relationship between Ct and patient sociodemographic and clinical characteristics, as well as the relationship between Ct and disease severity, to better understand the role of Ct in estimating patients' risk for adverse outcomes.

2 | METHODS

2.1 | Patient population

We analyzed data from all symptomatic patients age 18 and older who presented to the Emergency Department at Tufts Medical Center, a tertiary care facility in Boston, Massachusetts, between March 20 and July 13, 2020. The study included all patients who tested positive for SARS-CoV-2 on a nasopharyngeal (NP) swab. Ct values, as well as patient demographic and clinical characteristics, were collected to study patients through the review of patient's medical records.

2.2 | SARS-CoV-2 PCR testing

Testing was performed using the Abbott M2000 SARS-CoV-2 assay. This assay is an rRT-PCR dual-target assay for the RNA polymerase (RdRp) and N genes of the SARS-CoV-2 virus[12] and has a positive cutoff value of 31.5. This assay has been demonstrated to have high sensitivity (93%) and specificity (100%) for SARS-CoV-2[13] and allows for the determination of Ct values. This assay utilizes negative and positive controls to evaluate the validity of testing,[12] and all providers performing the testing were trained in appropriate NP swab collection.

2.3 | Study definitions

Demographic characteristics including age, sex, race, and ethnicity were extracted from the electronic medical record (EMR), as were clinical characteristics including time since symptom onset, disease severity, and the presence of medical comorbidities. Disease severity was classified according to the National Institutes of Health (NIH) clinical spectrum categories[14] and categorized as asymptomatic, mild, moderate, severe, or critical as assessed by an independent reviewer. Disease severity was categorized for two time points: time of testing and point of greatest clinical severity. Time since system onset was extrapolated from the EMR based on documentation of patient report. Medical comorbidities were stratified into three groups: (1) no comorbidities (2) 0–2 comorbidities or (3) >2 comorbidities.

2.4 | Statistical analysis

Differences in Ct values by sex, age, race, and ethnicity were compared by Kruskal–Wallis testing. Univariable and multivariable logistic regression analyses were used to evaluate the relationship between time since symptom onset and Ct, and to analyze Ct as a predictor for severe disease at the time of testing or at the point of greatest severity in the patient's disease course. Multivariable adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated in a standard manner. Model fit was assessed using the C statistic. For all analyses, p values of <0.05 were considered statistically significant. All statistical analysis was performed using SAS 9.4 statistical software.

3 | RESULTS

A total of 256 patients satisfied our study criteria. The mean age of the study population was 55.5 years, 45% were women, 40% were White, 22% were Black, 16% were Asian and 17% were Hispanic. Medical comorbidities were present in 69.5% of the population, with 23% having more than two medical comorbidities. In terms of disease severity, 8.6% were asymptomatic, 21.9% experienced mild disease, 33.6% had moderate disease, 12.9% had severe disease, and 23% experienced critical disease. Median time since symptom onset was 6 days.

3.1 | Ct values and patient demographic characteristics

There were no significant differences in Ct values according to patient's age, sex, race, or ethnicity (Table 1). There was a trend toward higher Ct values in patients identifying as Hispanic and lower Ct values in older (>65 years) patients.

TABLE 1 Relationship between cycle threshold and demographic characteristics.[a]

Variable	Category	N	Median	Lower–upper quartile	p
Sex	Female	115	16.9	10.5–21	0.4
	Male	141	16.7	12.6–21.5	
Age group	18–49 years	85	16.8	12.1–21.0	0.6
	50–64 years	84	17.6	12.1–21.4	
	65+	85	15.3	10.8–20.6	
Race	Asian	40	15.3	12.6–19.1	0.3
	Black	57	17.3	11.6–22	
	Other	57	18.8	14.1–20.9	
	White	102	16.3	10–21.0	
Ethnicity	Hispanic or Latino	43	18.8	15.1–20.8	0.1
	Not Hispanic or Latino	213	16.3	10.8–21.1	

[a]Compared vis Kruskal–Wallis test.

3.2 | Ct values and relationship to disease severity, presence of symptoms, and time since symptom onset

Table 2 shows full results from univariable and multivariable modeling. After controlling for sex, age, race, ethnicity, and medical comorbidities, Ct values increased with longer time since symptom onset ($p < 0.001$). Using Ct as a predictor of disease severity at the time of testing, the odds of severe disease increased with higher Ct (OR: 1.05, 95% CI: 1.0–1.11) even after controlling for time since symptom onset and patient demographic characteristics (C statistic = 0.759). This association was also seen when looking at disease at the worst point in patient illness, although the findings were not significant (OR: 1.03, 95% CI: 0.98–1.08, C statistic = 0.787). Restricting the study population to the patients admitted after testing, a similar association was seen between Ct and disease severity in the multivariable model (OR: 1.06, 95% CI: 0.99–1.02), although this association was not statistically significant.

4 | DISCUSSION

The results of the present study suggest that Ct is not associated with patient demographic characteristics including age, sex, race, and ethnicity. Ct values increased with increasing time from symptom onset, and increased odds of severe disease were seen with increasing Ct values.

A previous observational study conducted during the first phase of the pandemic in Greece[8] demonstrated that those who were older had lower Ct values at the time of diagnosis than younger individuals. On the other hand, a prospective study investigating transmission among household members with a SARS-CoV-2 exposure failed to find an association between Ct and age, race, or ethnicity, consistent with our findings.[9] However, the relationship of Ct values with race and ethnicity is an area that warrants continued study given observed disparities in disease outcomes due to SARS-CoV-2 infection.[15,16]

In the present study, greater disease severity was associated with higher values of Ct, which suggests lower viral loads in sicker patients. While prior studies have shown that lower Ct results were associated with an increased odds of severe COVID-19,[7,8,17] our study failed to confirm these findings. This may be reflective of the small sample size included in our study, although previous studies had similar population sizes. A previous observational study conducted in New York City during the initial phase of the pandemic[11] demonstrated similar findings to ours and hypothesized that this may reflect the association between time from onset of infection and increased disease severity. This is because viral load peaks during the presymptomatic stage of the disease course and declines to become undetectable by Days 18–21.[18] Consistent with this, patients for whom hospitalization was required were found to have presented later in the disease course in the New York study.[11] Another study conducted at a large academic center also found no association between Ct values and disease severity or mortality, which was consistent with our findings.[10] In our study while we did find, like others, increasing Ct values as time since symptom onset increased,[7,8] the relationship between Ct and disease severity persisted even after controlling for time since symptom onset and patients' demographic characteristics. Our findings may be reflective of error in accurately ascertaining the timing of symptom onset due to the retrospective nature of the study.

Our study provides insights into the patient characteristics and outcomes associated with SARS-CoV-2 infection during a time of rapidly changing viral transmission. Our study has several limitations, however, that need to be kept in mind when interpreting the results. It was conducted over a relatively short period of time at a single institution and included a relatively small number of patients. However, our patient population is unique in that the study institution serves a larger Asian American population than those in other studies, providing new information on this race/ethnicity. Another limitation is that to maintain consistency in testing results, we only analyzed Ct values from one diagnostic testing platform; therefore, findings may not be applicable to all SARS-CoV-2 assays. However, this testing platform has been well-validated[19]; thus, we suspect that our findings may also be applicable to other diagnostic assays.

In conclusion, we found that SARS-CoV-2 viral loads as determined by Ct values generated with rRT-PCR viral assays increase with time since symptom onset, but are not significantly different among men and women of different ages, races, or ethnicities. Unexpectedly, patients with higher Ct values

TABLE 2 Univariable and multivariable model of Ct and symptoms

Ct association with clinical characteristics	Effect variable	Univariable model	p	Multivariable model[a]	p
Time from symptom onset[b]	Beta[c]	0.32 (0.08)	<0.001	0.34 (0.08)	<0.01
Severe disease at testing[d]	Odds ratio[e]	1.05 (1.01–1.1)	0.03	1.05 (1.0–1.11)	0.05
Severe disease at worst point	Odds ratio[e]	1.02 (0.98–1.06)	0.3	1.03 (0.98–1.08)	0.3
Severe disease at testing (hospitalized)	Odds ratio[e]	1.06 (1.01–1.12)	<0.05	1.06 (0.99–1.12)	0.08

[a]Multivariable model controlled for sex, age, race, ethnicity, time since symptom onset, and medical comorbidities.

[b]Linear regression with outcome of cycle threshold; time since symptom onset as predictor variable.

[c]Beta coefficient and standard error.

[d]Logistic regression with outcome of severe disease and cycle threshold as a predictor variable.

[e]Odds ratio and 95% confidence interval.

demonstrated an increased risk of severe disease, even when controlling for time from symptom onset. Continued study will allow us to better comprehend the complex factors that contribute to the risk for severe outcomes due to SARS-CoV-2 infection.

AUTHOR CONTRIBUTIONS

Jessica Penney and Amanda Jung contributed equally to the manuscript. Benjamin Koethe performed statistical analyses. All authors (Jessica Penney, Amanda Jung, Benjamin Koethe, and Shira Doron) contributed to the many revisions and approved the final version for submission.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved by Tufts Health Sciences Institutional Review Board and granted exempt status.

SUMMARY

Cycle threshold (Ct) values increased with time since symptom onset and did not differ by age, gender, race, or ethnicity despite differences in risk for severe disease. Increasing Ct values were associated with increased odds of severe disease.

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REFERENCES

1. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. Accessed June 28, 2020. https://covid19.who.int/
2. Rambaut A, Holmes EC, O'toole Á, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat Microbiol*. 2020;5:1403-1407. doi:10.1038/s41564-020-0770-5
3. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA*. 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775
4. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585
5. Liang W, Liang H, Ou L, et al. Development and validation of a clinical risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19. *JAMA Intern Med*. 2020;180(8):1081-1089. doi:10.1001/jamainternmed.2020.2033
6. Tom MR, Mina MJ. To interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis*. 2020;71(16):2252-2254. doi:10.1093/cid/ciaa619
7. Magleby R, Westblade LF, Trzebucki A, et al. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis*. 2020;73:4197. doi:10.1093/cid/ciaa851
8. Maltezou HC, Raftopoulos V, Vorou R, et al. Association between upper respiratory tract viral load, comorbidities, disease severity, and outcome of patients with SARS-CoV-2 infection. *J Infect Dis*. 2021;223(7):1132-1138. doi:10.1093/infdis/jiaa804
9. Salvatore PP, Dawson P, Wadhwa A, et al. Epidemiological correlates of polymerase chain reaction cycle threshold values in the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2021;72(11):e761-e767. doi:10.1093/cid/ciaa1469
10. Camargo JF, Lin RY, Komanduri KV. Lack of correlation between the SARS-CoV-2 cycle threshold (C_t) value and clinical outcomes in patients with COVID-19. *J Med Virol*. 2021;93(10):6059-6062. doi:10.1002/jmv.27171
11. Argyropoulos KV, Serrano A, Hu J, et al. Association of initial viral load in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients with outcome and symptoms. *Am J Pathol*. 2020;190(9):1881-1887. doi:10.1016/j.ajpath.2020.07.001
12. Abbott RealTime SARS-CoV-2 [EUA Package Insert]. 2020. Abbott Molecular Inc. Accessed July 13, 2021. https://www.fda.gov/media/136258/download
13. Degli-Angeli E, Dragavon J, Huang ML, et al. Validation and verification of the Abbott RealTime SARS-CoV-2 assay analytical and clinical performance. *J Clin Virol*. 2020;129:104474. doi:10.1016/j.jcv.2020.104474
14. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Accessed July 13, 2021. https://www.covid19treatmentguidelines.nih.gov/
15. Adhikari S, Pantaleo NP, Feldman JM, Ogedegbe O, Thorpe L, Troxel AB. Assessment of community-level disparities in coronavirus disease 2019 (COVID-19) infections and deaths in large US metropolitan areas. *JAMA Netw Open*. 2020;3(7):e2016938. doi:10.1001/jamanetworkopen.2020.16938
16. Liao TF, De Maio F. Association of social and economic inequality with coronavirus disease 2019 incidence and mortality across US counties. *JAMA Netw Open*. 2021;4(1):e2034578. doi:10.1001/jamanetworkopen.2020.34578
17. Pujadas E, Chaudhry F, McBride R, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med*. 2020;8(9):e70. doi:10.1016/S2213-2600(20)30354-4
18. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med*. 2020;26(5):672-675. doi:10.1038/s41591-020-0869-5
19. Hirschhorn JW, Kegl A, Dickerson T, et al. Verification and validation of SARS-CoV-2 assay performance on the Abbott *m*2000 and Alinity *m* Systems. *J Clin Microbiol*. 2021;59(5):e03119-20. doi:10.1128/JCM.03119-20

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