

RESEARCH ARTICLE

SARS-CoV-2 PCR cycle threshold at hospital admission associated with patient mortality

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Abstract

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cycle threshold (Ct) has been suggested as an approximate measure of initial viral burden. The utility of cycle threshold, at admission, as a predictor of disease severity has not been thoroughly investigated.

Methods and findings

We conducted a retrospective study of SARS-CoV-2 positive, hospitalized patients from 3/26/2020 to 8/5/2020 who had SARS-CoV-2 Ct data within 48 hours of admission ($n = 1044$). Only patients with complete survival data, discharged ($n = 774$) or died in hospital ($n = 270$), were included in our analysis. Laboratory, demographic, and clinical data were extracted from electronic medical records. Multivariable logistic regression was applied to examine the relationship of patient mortality with Ct values while adjusting for established risk factors. Ct was analyzed as continuous variable and subdivided into quartiles to better illustrate its relationship with outcome. Cumulative incidence curves were created to assess whether there was a survival difference in the setting of the competing risks of death versus patient discharge. Mean Ct at admission was higher for survivors (28.6, SD = 5.8) compared to non-survivors (24.8, SD = 6.0, $P < 0.001$). In-hospital mortality significantly differed ($p < 0.05$) by Ct quartile. After adjusting for age, gender, BMI, hypertension and diabetes, increased cycle threshold was associated with decreased odds of in-hospital mortality (0.91, CI 0.89–0.94, $p < 0.001$). Compared to the 4th Quartile, patients with Ct values in the 1st Quartile (Ct < 22.9) and 2nd Quartile (Ct 23.0–27.3) had an adjusted odds ratio of in-hospital mortality of 3.8 and 2.6 respectively ($p < 0.001$). The discriminative ability of Ct to predict inpatient mortality was found to be limited, possessing an area under the curve (AUC) of 0.68 (CI 0.63–0.71).

Competing interests: NO authors have competing interests.

Conclusion

SARS-CoV-2 Ct was found to be an independent predictor of patient mortality. However, further study is needed on how to best clinically utilize such information given the result variation due to specimen quality, phase of disease, and the limited discriminative ability of the test.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 is a novel betacoronavirus that first appeared in Wuhan, Hubei Province, China in late December 2019 [1]. This virus has led to a global pandemic with over 23 million people infected worldwide with an overall mortality rate between 1.4% and 5% [2, 3]. It was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 [4]. While scientists, clinicians and researchers continue to grapple with the infection, the continuous in-flow of clinical data is helping to guide diagnostic, treatment and prognostic characteristics of the disease.

COVID-19 has wide-ranging clinical presentation, with 80% of cases being mild, 15% developing lower respiratory tract disease such as pneumonia, and less than 5% developing severe illness [5]. For those who progress to severe disease, the clinical course is insidious, with mild initial illness progressing to major complications in the second week [5]. The requirement for mechanical ventilation ranges from 18 to 33%; approximately 20% of hospitalized patients die of disease [6–10]. Multiple factors have been studied in association with disease severity, such as D-dimer, lymphopenia, obesity, hypertension and diabetes mellitus (DM) and there is still a continuous search for a reliable marker to predict disease aggressiveness [11–16].

Real-time reverse-transcriptase PCR (RT-PCR) has served as a major modality in diagnosing SARS-CoV-2 infection. Given that PCR amplifies a target stretch of nucleic acid exponentially, samples which begin the reaction with more abundant target material, will produce a detectable signal earlier than samples with lower target abundance. The cycle threshold value (Ct) derived from a sample is essentially a measure of the amplification required for the target viral gene to cross a threshold value and is inversely related to the viral load [17]. Kawase et al. have defined Ct as the cycle number when the sample fluorescence exceeds a set above the calculated background fluorescence [18]. In fact, while many are familiar with the term “viral load” for the quantification of certain viruses, this value is most commonly not measured directly but interpolated from a standard curve generated using the identified Ct values found from samples of known concentration. The Ct value is the inverse of viral load and approximately every 3.3 increase in Ct value reflects a 10-fold reduction in starting material [19]. There is limited literature exploring the association of Ct and disease mortality, and there has been variability in the findings [10, 17, 20, 21]. The objective of this study is to examine the relationship between SARS-CoV-2 cycle threshold at hospital admission and its relationship to patient outcome.

Methodology

In this retrospective observational study, we included all hospitalized patients at Montefiore Medical Center between 3/26/2020 and 8/5/2020 with a positive SARS-CoV-2 nasopharyngeal swab specimen on the Panther Fusion System (Hologic, Inc.) that was collected within 48

hours of admission. Subjects under the age of 18, current inpatients, those with initial SARS-CoV-2 testing on the Fusion system >48 hours after admission, or missing BMI were excluded from the analysis (Fig 1). The patient information and data including test results and comorbidities were collected retrospectively from electronic medical records. Vital sign data included in the study, represented the first recorded vitals in the hospital records during admission and the biochemical and other parameters were indexed as the closest result within 48 hours to SARS-CoV-2 testing. The study was approved by the Albert Einstein College of Medicine Institutional Review Board. Due to the retrospective nature of the study informed consent was waived.

Viral testing was performed on the Panther Fusion System (Hologic, Inc.) RT-PCR platform, which has received Emergency Use Authorization with the Food and Drug Administration (FDA). All testing was performed according to the manufacturer's instructions. The basic steps of the assay include sample lysis, nucleic acid capture, elution transfer, and multiplex RT-PCR where analytes are simultaneously amplified and detected. Results are reported as positive or negative depending on detectable amplification. The instrument also generates a Ct value. For the purpose of this study, Ct was divided into quartiles (Q1, Q2, Q3 and Q4) for grouping samples and studying the significance of viral load as an indirect marker. Low Ct corresponded to higher viral load.

Statistical analysis

Demographic and baseline group differences were assessed using chi-square tests, 2-sample Student t-tests, and for non-normally distributed data, the Mann-Whitney U test. The correlation between variables was assessed using the Kendall rank correlation method due to non-parametric data. The relationship between in-hospital mortality, cycle quartile, clinical risk factors, and biomarkers was modeled using logistic regression. Model covariate selection was based on literature review of identified SARS-CoV-2 survival risk factors. Model fit was assessed using the Hosmer and Lemeshow test which found $p > 0.05$, thus not detecting evidence of poor fit. Multicollinearity was evaluated using variance inflation factors. Model discriminative ability was validated by calculating bootstrapped AUC. Cumulative incidence curves were created to assess whether there was a survival difference by cycle threshold quartile. This approach was used to account for the competing risks of in-hospital mortality versus patient discharge. A sensitivity analysis was carried out which assessed for patterns in missing data, study result sensitivity to covariate selection, and time period selected (S1 Fig, S1–S3 Tables). Analyses were performed using R version 3.6.2. A p -value < 0.05 was considered statistically significant.

Results

A total of 1,044 patients met study inclusion criteria, of these 774 (74.1%) survived to discharge and 270 (25.9%) expired (Fig 1). In our cohort, 55.6% (580) patients were males and 44.4% (464) females with a mean age of 65.2 years (SD 15.37) and a mean BMI of 29.6 (SD 7.4). A history of hypertension (HTN) and Diabetes (DM) was present in 64.1% (629) and 40.8% (400), respectively. The majority of patients had group O blood-type, 46.5% (410), followed by group A blood-type, 31.6% (278).

Ct at admission was positively correlated with patient survival ($r = 0.22$, $p < 0.001$) (Fig 2). To better illustrate the magnitude of the effect, Ct was divided into quartiles (Q1, Q2, Q3 and Q4). The Q1 group consisted of cycle numbers < 22.9 , Q2 was cycle numbers between 23.0 and 27.3, Q3 was between 27.4 and 32.8 and Q4 was > 32.9 . It was noted that mortality was significantly different between the four groups, with highest mortality in those with lowest Ct

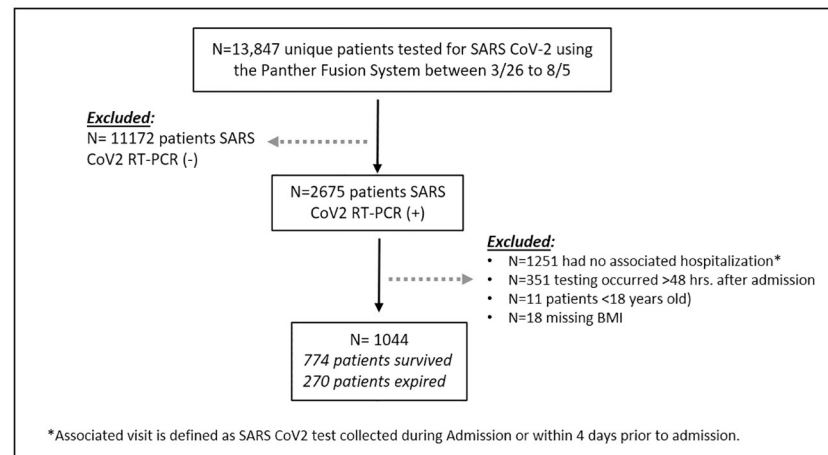


Fig 1. Study cohort.

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(Q1 = 41.4%) and lowest in the group with highest Ct (Q4 = 13.2%) ($p < 0.001$) (Table 1). The association of cycle quartile and patient mortality held when stratified across age groups (Fisher's exact test $p < 0.05$ for all groups) (Fig 3). The cycle quartile was compared between age groups and gender (Fig 4). The cumulative incidence of hospital discharge varied across the cycle quartile (Gray's Test $P < 0.001$), as did the patient mortality (Gray's test $P < 0.001$) (Fig 5).

A multivariate logistic model was created describing the impact of cycle quartile, patient age, gender, BMI, and the comorbidities of hypertension and diabetes on patient survival. With the fourth cycle quartile (Q4) set as reference the adjusted odds of patient mortality increased as cycle quartile and correspondingly cycle number decreased, becoming significant in Q2 (aOR 2.63, CI 1.6–4.2, $P < 0.001$) and Q1 (aOR 3.85, CI 2.3–6.2, $p < 0.001$) (Table 2). Male gender was found to confer a heightened risk of COVID-19 in-hospital mortality (aOR 1.88, CI 1.37–2.59, $p < 0.01$). Likewise, increased age, BMI, and a history of diabetes were all found to increase adjusted odds of in hospital mortality. Interestingly comorbid hypertension was found significant in univariable analysis but lost significance in the multivariable model.

The use of cycle threshold as a reported parameter predictive of inpatient mortality is limited by variation in sample collection, sample run variation, and the lack of a high-performing cutoff value (Figs 6 and 7). The variation in optimal cutoff was assessed by bootstrapping with 1000 replications with ideal cutpoint determined using Youden's index. The cutpoint which maximized Youden's statistic was found to be 26 (CI 26–27). At this cutpoint, the testing set (out-of-bag) sensitivity was found to be 0.65 (CI 0.58–0.72) and specificity was 0.64 (CI 0.57–0.69). The area under the curve (AUC) was 0.68 (CI 0.63–0.71), thus the discriminative ability of the test to predict inpatient mortality was limited.

Discussion

The coronavirus is an enveloped, positive-sense, single-stranded RNA virus. It is known to infect a variety of host species and is divided into four genera; α , β , γ , and δ based on the genomic structure. The COVID-19 virus belongs to the β group and consists of four structural proteins; Spike (S), membrane (M), envelop (E) and nucleocapsid (N) and the virus has characteristic spikes (S glycoprotein) radiating from the viral surface [22]. The genes for the N and E proteins are used as the targets for amplification in the various rRT-PCR assays combined with the open reading frame 1 (ORF1) ab, and the RNA-dependent RNA polymerase (RdRP)

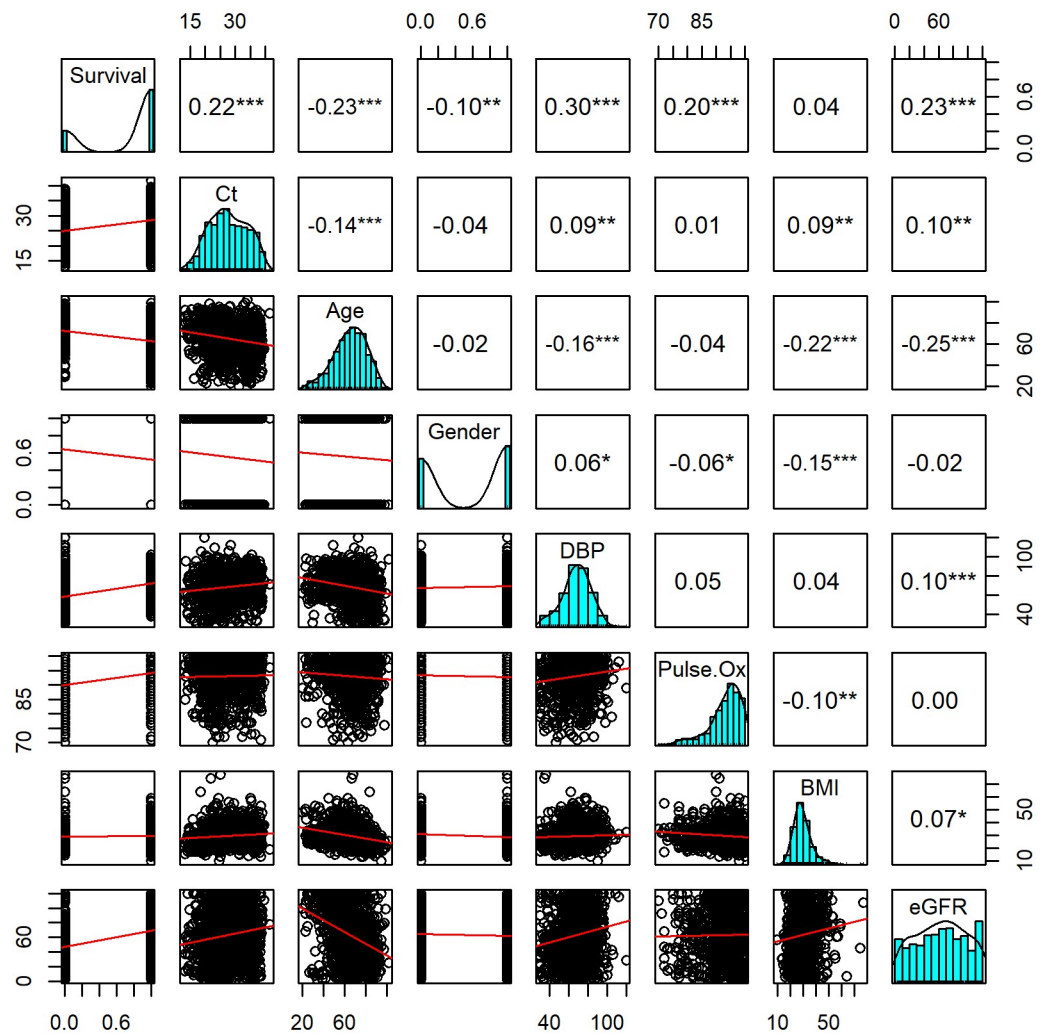


Fig 2. Pairwise comparisons of in hospital mortality and patient characteristics at presentation. Kendall correlation matrix plot shows correlation coefficients on one side (top and right) and bivariate scatter plots, along with linear regression lines of best fit on the other side (bottom and left). The top and right panels also include asterisks, which show significance of each pairwise comparison: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The dividing diagonal boxes demonstrate the distribution of each variable studied.

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gene [23]. Studies on viral structure and its receptors have helped improve understanding of the nature of disease and interpretation of test results. While the CDC assay has probes for nucleocapsid (N) gene targets and human RNase P gene (RP), the Panther Fusion platform works by amplifying and detecting two conserved regions of the ORF1ab gene. The two regions are not differentiated and amplification of either or both leads to a signal and positive result. Fung et al. compared the limit of detection (LOD) for various assays and reported it to be between 85–499 copies/mL for CDC assays depending on extraction instrument and thermocycler used and 74 copies/mL for Panther fusion [24]. Liberman et al. compared the Ct for CDC assay and Panther fusion before and after FDA approval. They reported slightly lower sensitivity for the Panther Fusion platform and the discordant results were either inconclusive (one target of two detected in CDC) or had high average Ct value (>37) in CDC test [25].

The clinical severity of COVID-19 infection is highly-variable, thus identifying patients at risk for aggressive disease is critical for triage and early management. While BMI, history of

Table 1. Study characteristics by COVID-19 PCR cycle quartile.

	Overall Cohort	Cohort characteristics stratified by PCR Cycle Quartile				p value
		Q1 (<22.9)	Q2 (23.0–27.3)	Q3 (27.4–32.8)	Q4 (>32.9)	
n (%)	1044	263	263	260	258	
Mortality, n (%)	270 (25.9)	109 (41.4)	84 (31.9)	43 (16.5)	34 (13.2)	<0.001
LOS, median [IQR]	6.0 [3.0, 11.0]	6.0 [3.0, 12.0]	6.0 [3.0, 9.2]	6.0 [3.0, 9.7]	6.0 [4.0, 9.7]	0.33
Male, n (%)	580 (55.6)	153 (58.2)	154 (58.6)	134 (51.5)	139 (53.9)	0.29
Age, mean (SD)	65.2 (15.3)	69.4 (15.0)	66.6 (14.6)	62.6 (14.3)	62.2 (17.5)	0.52
BMI, mean (SD)	29.6 (7.4)	27.8 (7.6)	30.3 (7.8)	30.2 (6.8)	30.1 (7.1)	<0.001
eGFR, mean (SD)	63.1 (34.5)	55.9 (33.9)	60.7 (35.6)	67.8 (33.4)	69.0 (33.2)	<0.001
LDH, median [IQR]	403 [303, 548]	373 [270, 537]	393 [306, 549]	419 [321, 582]	413 [306, 538]	0.03
Initial PO ₂ , mean (SD)	93.0 (5.8)	94.4 (7.5)	94.7 (7.6)	94.8 (6.6)	94.6 (7.2)	0.48
Initial DBP*, mean (SD)	68.4 (14.9)	66.2 (15.8)	66.3 (15.2)	70.3 (14.2)	70.8 (13.6)	<0.001
Hx of DM [Yes], n (%)	400 (40.8)	113 (44.5)	98 (38.0)	100 (39.7)	89 (41.0)	0.49
Hx of HTN [Yes], n (%)	629 (64.1)	180 (70.9)	163 (63.2)	157 (62.3)	129 (59.4)	0.06
ABO Type, n (%)						0.24
Type A	278 (31.6)	85 (36.6)	77 (33.6)	57 (26.9)	59 (29.2)	
Type AB	41 (4.7)	8 (3.5)	11 (4.8)	11 (5.2)	11 (5.3)	
Type B	152 (17.3)	29 (12.6)	43 (18.8)	43 (20.3)	37 (17.7)	
Type O	410 (46.5)	109 (47.2)	98 (42.8)	101 (47.6)	102 (48.8)	

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hypertension, and DM have been well investigated, there are limited studies which have evaluated the utility of rRT-PCR Ct as a predictor of disease severity [10, 26]. Although it is difficult to utilize raw Ct values as a substitute for viral burden without the production of an appropriate standard curve or normalization to an internal housekeeping gene, attempts have been made to examine just this utility [27]. While the efforts at producing such normalization and quantification are praiseworthy, the production of a standard curve for quantification may not represent any additional benefit over utilizing the Ct values from which the viral loads themselves are determined. Additionally, a calculated definitive viral burden may not be accurate when an inhibitor is present within an individual sample or when PCR efficiency is affected by variants within the viral genome.

Previous studies which have looked at Ct or viral loads for disease aggressiveness have found differing results, which may be attributed to variability within the temporal course of infection [10, 21, 28]. Some studies have reported that peak load of the virus in upper respiratory tract specimens was expected during early stages of infection and in the pre-symptomatic stage, while others have found the peak to be approximately two weeks after the onset of symptoms even extending into the 3rd or 4th week of illness [29–32].

Zheng et al. described increased viral load in respiratory samples in patients with a more aggressive disease course as compared to those with milder disease and therefore found that it could be used as a possible indicator of prognosis [30]. Similar results have been reported by Liu et al [33]. This correlation was not seen in relation to the viral load in stool samples [30]. Chen et al. reported that patients in the intensive care unit (ICU) continued to remain positive for COVID-19 infection longer than those who were not in the ICU [34]. Rao et al. in their systematic review of 18 studies reported an association between the viral load (or cycle number) and clinical outcome [17]. There was only one among 18 studies reviewed that looked at cycle number and mortality and found lower cycle number value associated with increased risk of mortality [17, 32]. Our results demonstrate a similar pattern as we found Ct values correlated inversely with mortality and low Ct increased the odds ratio for mortality compared to higher Ct.

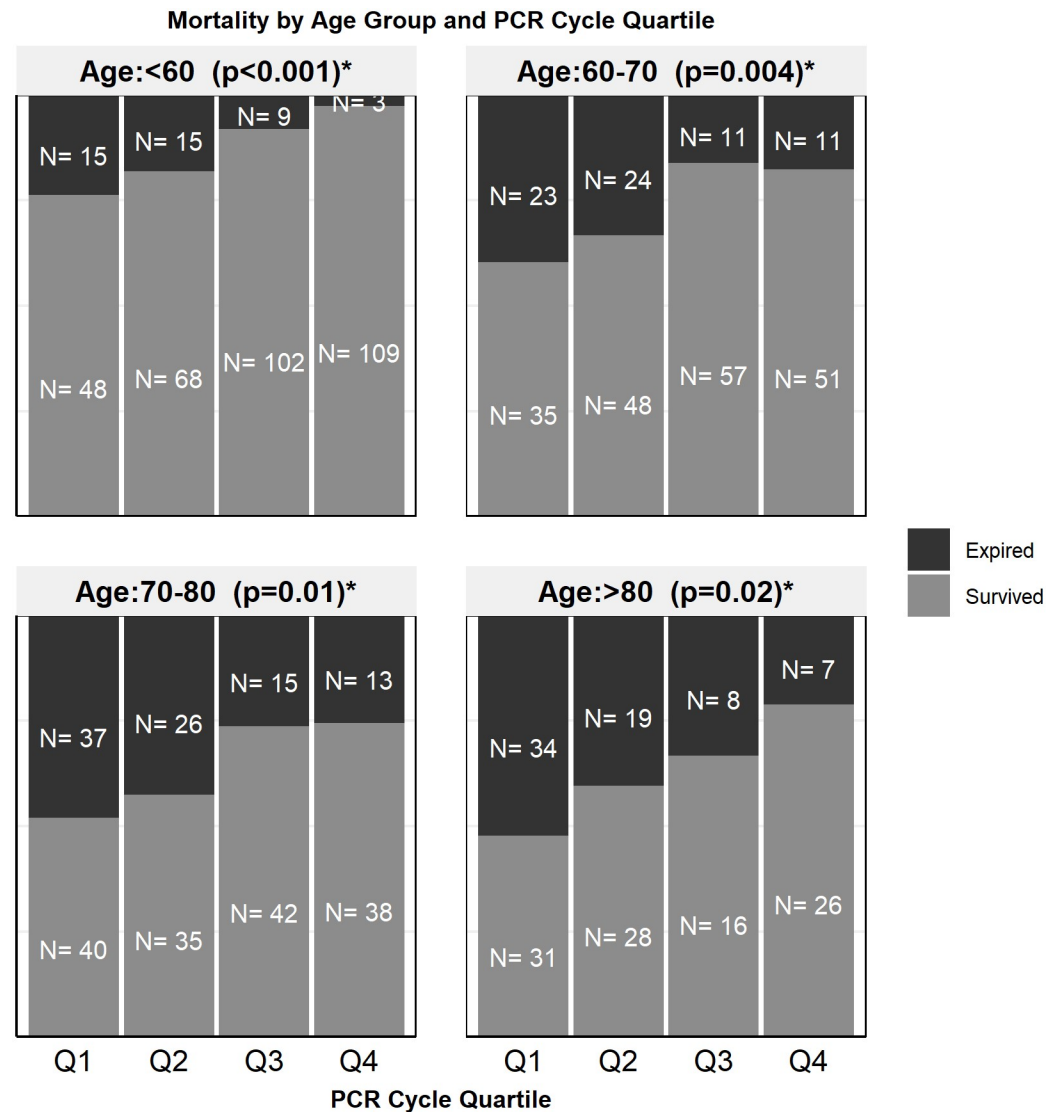


Fig 3. Stacked bar graph of mortality by PCR cycle quartile and age group. *Fisher's exact test used.

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According to Rao et al. 73% of the studies which looked at Ct in hospitalized patients found an association between Ct and disease aggressiveness, however, none of the studies which identified this association involved non-hospitalized patients [17]. Argyropoulos et al. in their research did report higher viral loads in non-hospitalized patients with an inverse relation between viral load and duration of symptoms and its severity [21]. The available literature suggests that recruitment of hospitalized patients might be a potential bias of analyzing the more severely ill among the overall infected population. Argyropoulos et al. did not find any association between viral load and length of hospital stay [21]. In contrast, our results did show a difference in survival times by Ct value quartile.

Comparing our cohort of New York patients with findings by Magleby et al. it was noted that both showed a statistically significant difference between age groups and the Ct [10]. However, we also noted a difference related to male gender, which was not noted in their analysis [10]. Magleby et al. reported that patients with high viral loads were at increased risk of

COVID PCR Cycle Number and Mortality by Age and Gender

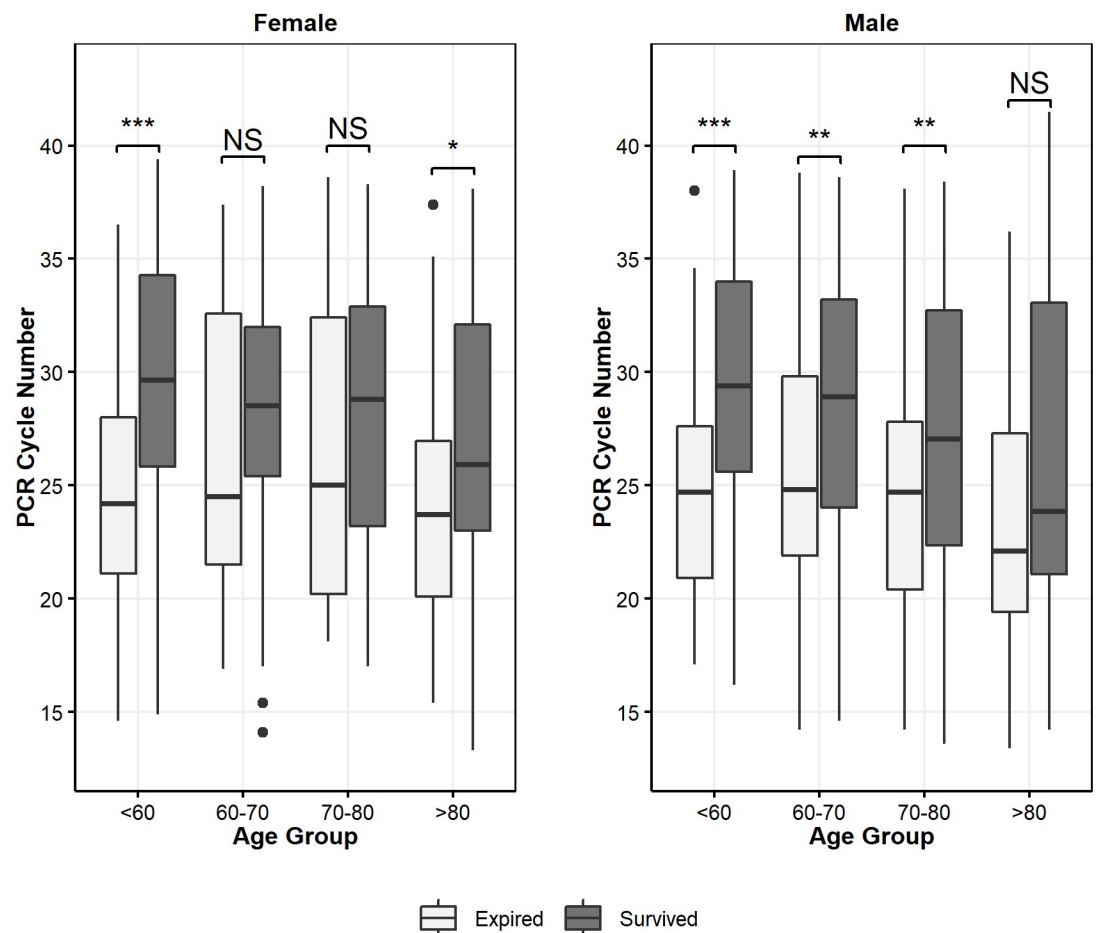


Fig 4. Cycle number and mortality by age and gender.

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myocardial infarction and acute kidney injury [10]. We reached a similar inference regarding GFR and the Ct (viral load) showing that patient with high viral load had poorer GFR. Rao et al. found elevated LDH corresponded to low cycle numbers (higher viral load) and reported it as an important marker which showed consistent results in all the four studies in which it was included [17]. LDH was significantly different between patients of the different Ct quartiles in our study as well. Contrary to studies which have found ABO blood group as an indicator of disease severity, we were not able to identify such a correlation [35].

Our study suggests that Ct can be used as an independent indicator of mortality—this corroborates previously reported data [29]. While Ct has been observed to be an important indicator of viral infection, it is influenced by the assay used and factors that can affect the amplification efficiency [35]. While studies like ours have helped identify independent markers which may in future help clinicians predict outcome, we are still cautious about its reliability for several important reasons. Firstly, most studies, including ours, have performed their analysis based on a single patient result. It is well understood that the testing is greatly limited by the quality of sample and its associated collection. This variability might be overcome

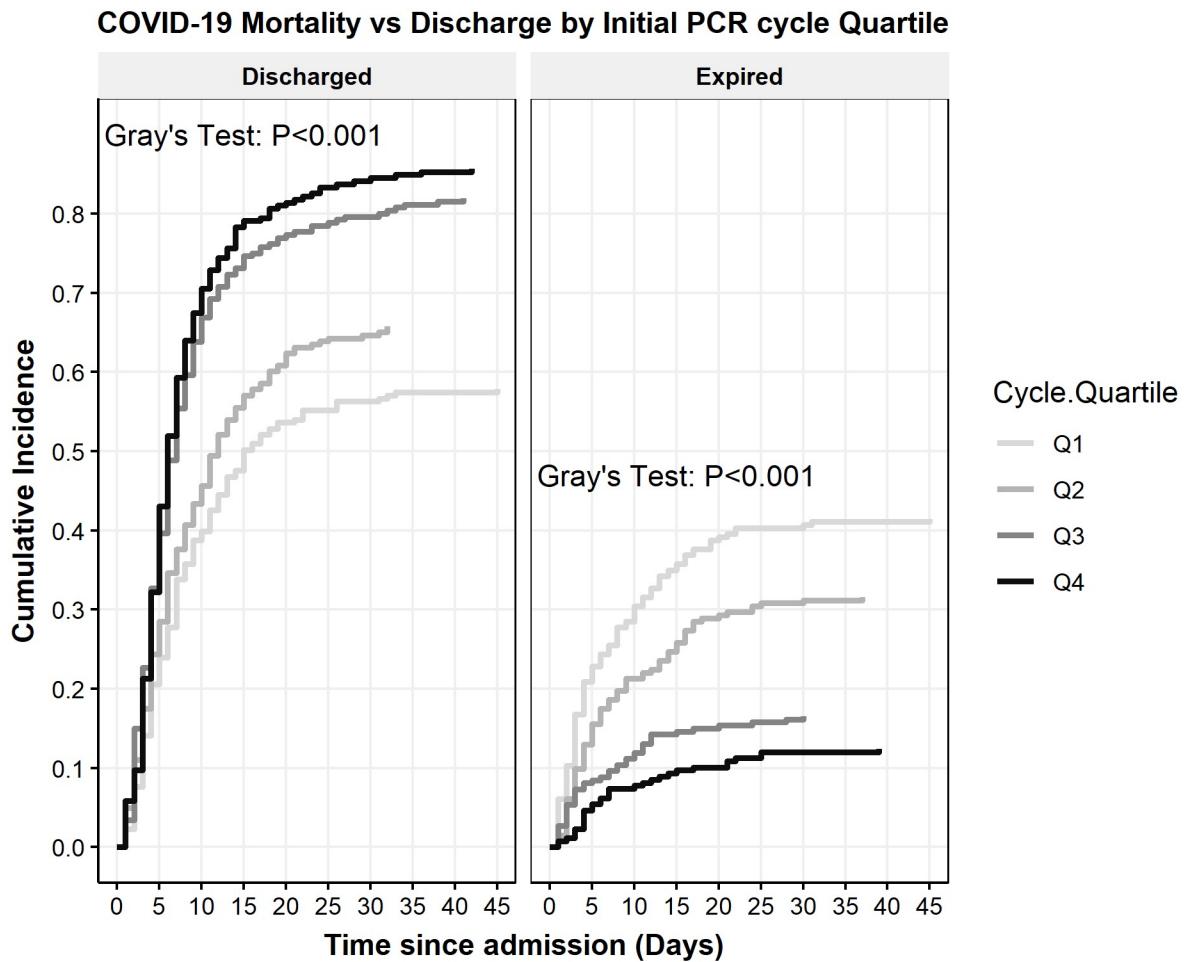


Fig 5. Cumulative incidence function of COVID-19 mortality vs discharge by initial PCR cycle threshold quartile.

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Table 2. Logistic regression table of in-hospital mortality.

In-Hospital Mortality		Expired	Survived	Odds ratio (univariable)	Odds ratio (multivariable)
Age	Mean (SD)	72.4 (12.9)	62.8 (15.4)	1.05 (1.04–1.06, p<0.001)	1.05 (1.04–1.06, p<0.001)
Cycle Quartile	Q4	34 (12.6)	224 (28.9)	-	-
	Q3	43 (15.9)	217 (28.0)	1.31 (0.8–2.1, p = 0.28)	1.29 (0.7–2.1, p = 0.33)
	Q2	84 (31.1)	179 (23.1)	3.09 (2.0–4.8, p<0.001)	2.63 (1.6–4.2, p<0.001)
	Q1	109 (40.4)	154 (19.9)	4.66 (3.0–7.3, p<0.001)	3.85 (2.3–6.2, p<0.001)
Gender	F	97 (35.9)	367 (47.4)	-	-
	M	173 (64.1)	407 (52.6)	1.61 (1.21–2.15, p = 0.001)	1.88 (1.37–2.59, p<0.001)
BMI	Mean (SD)	29.2 (8.2)	29.8 (7.2)	0.99 (0.97–1.01, p = 0.301)	1.03 (1.01–1.06, p = 0.006)
DM	No	139 (52.1)	442 (61.9)	-	-
	Yes	128 (47.9)	272 (38.1)	1.50 (1.13–1.99, p = 0.005)	1.38 (1.00–1.90, p = 0.048)
HTN	No	74 (27.7)	278 (38.9)	-	-
	Yes	193 (72.3)	436 (61.1)	1.66 (1.23–2.27, p = 0.001)	1.03 (0.72–1.47, p = 0.868)

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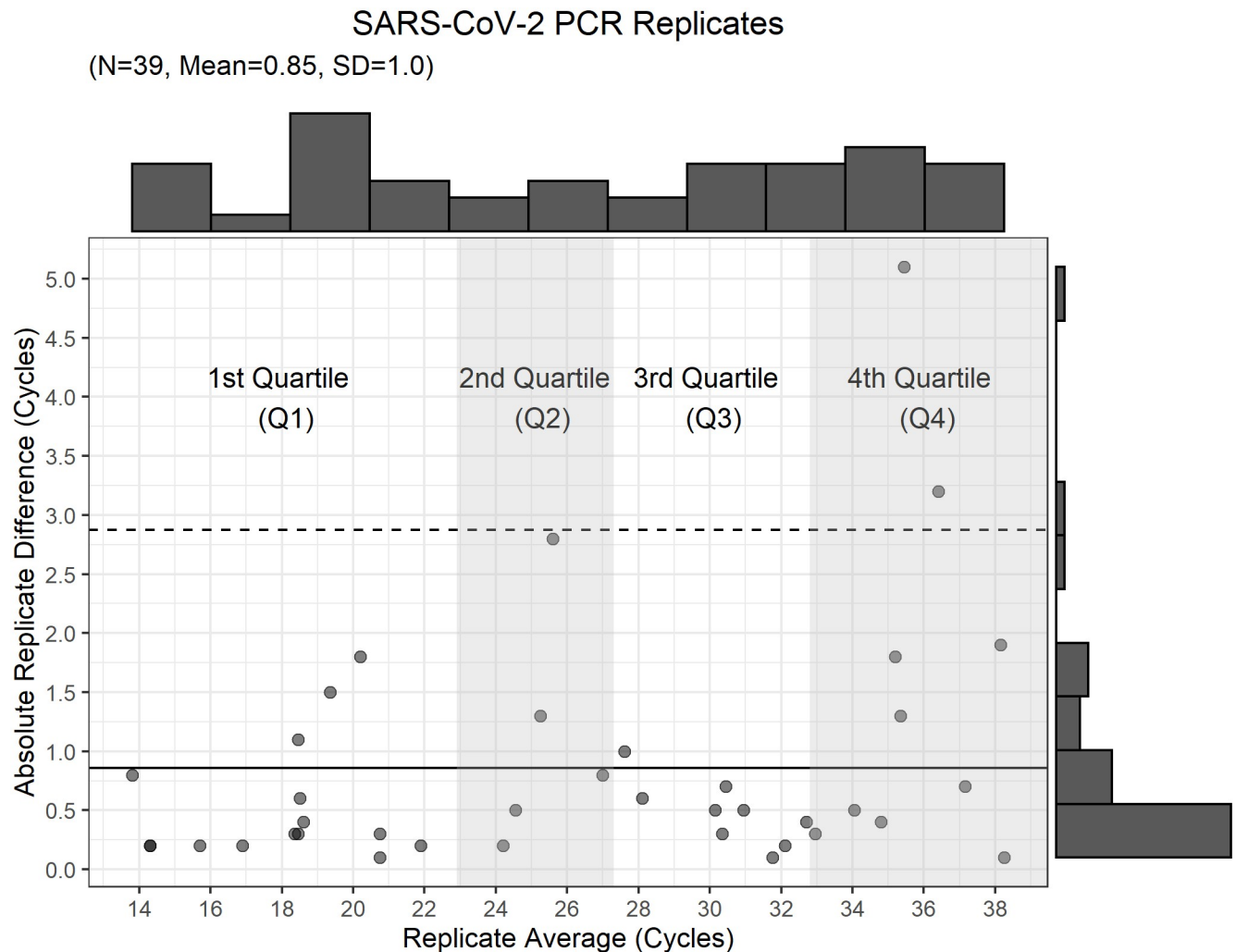


Fig 6. Scatterplot of averaged value of repeated samples versus absolute cycle number difference between replicates. The solid line indicates the mean absolute replicate cycle difference, and the dashed line marks 2 standard deviations from the mean. The top and right portions show a histogram of the axis variable.

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through multiple patient sampling to improve the robustness of Ct as a tool. Additionally, some studies have reported contrary results with respect to viral load and disease outcome which might imply that the temporal relation of viral load and the Ct interpretation may depend on stage of infection. Literature on Ct is also limited by the fact that studies have primarily focused on hospitalized patients and the larger cohort of non-hospitalized patients and those with milder disease forms of infection have not been analyzed thoroughly enough.

While our study included a large and diverse cohort of patients due to its geographical location, some of the limitations included inability to analyze significance of race/ethnicity due to missing data from the medical records. This study could not explore viral load in non-hospitalized patients, and mild or asymptomatic patients were not initially swabbed due to hospital policy.

Ct-based risk stratification or interpretation is also limited by the absence of absolute or constant Ct cut-off values. The Ct value ranges can vary widely by platform and are impacted by amplicon length, target region, PCR cycling protocols, and other reaction conditions which

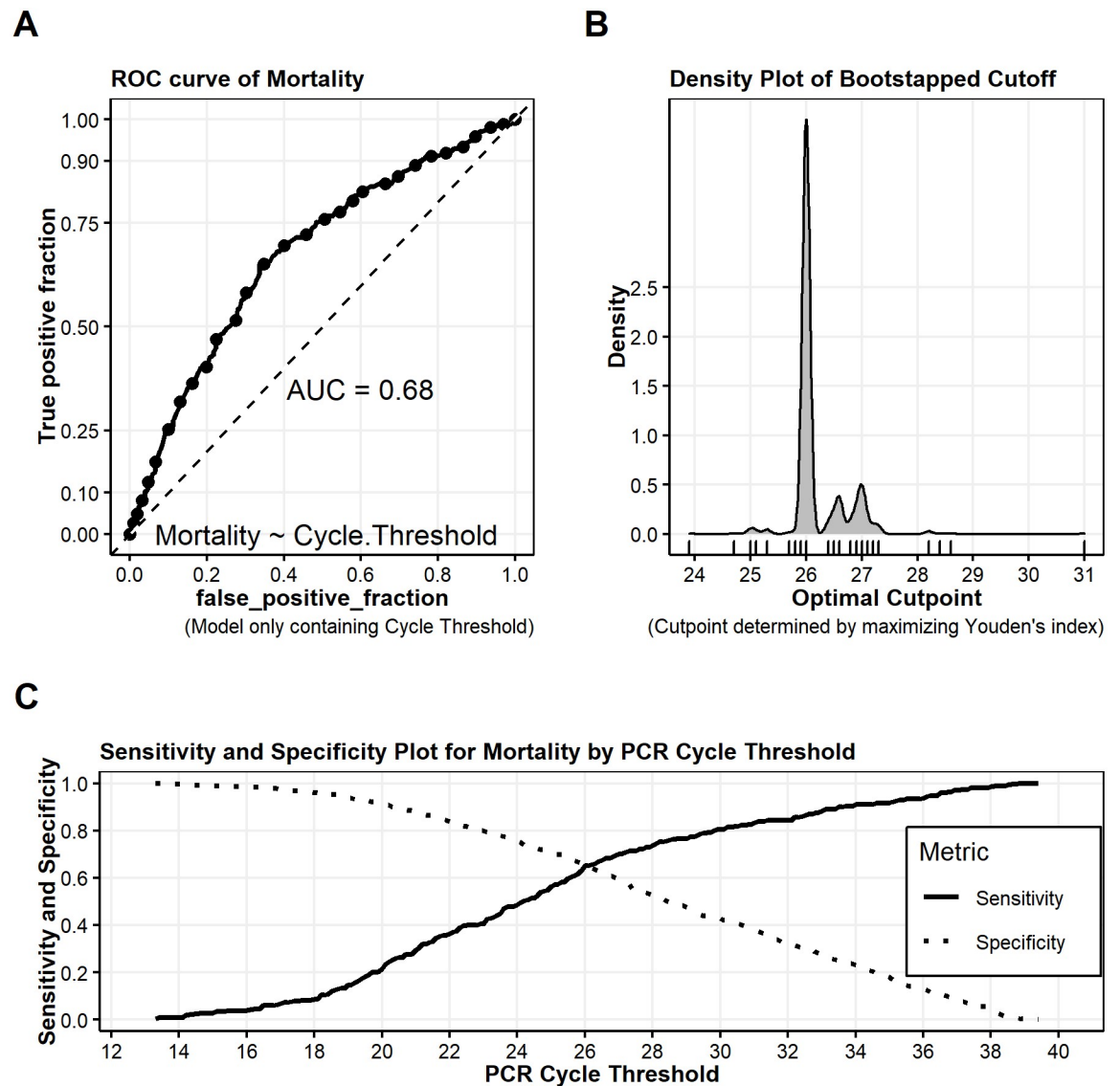


Fig 7. (Panel A): Receiver Operating characteristic (ROC) curve of Inpatient Mortality using a logistic model only containing the Cycle Threshold value; **(Panel B):** Density plot of optimal Cycle Threshold cutoffs using bootstrapping (1500 replications) and maximizing Youden's index; **(Panel C):** Sensitivity and Specificity Plot of Inpatient Mortality by PCR Threshold value.

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alter the overall PCR efficiency even when the same target gene is amplified [4]. Adopting the Ct value for clinical judgement is limited by the scope of error due to multiple factors including interpretation by the examiner [4]. Additionally, since testing is primarily upon nasopharyngeal swabs there is much greater variability from one collection to another in addition to variability between patient disease states [4]. Therefore, even with our promising results, the clinical applications of our findings would require further investigation to determine the ultimate value of Ct interpretation.

Conclusions

SARS-CoV-2 cycle threshold at admission was found to be an independent predictor of inpatient mortality. However, further study is needed on how to best clinically utilize such

information given the result variation due to specimen quality, phase of disease, and the limited discriminative ability of the test.

Supporting information

S1 Fig. (Left Panel): Bar chart of the proportions of missing values. (Right Panel): all existing combinations of missing values (Red) and non-missing values (Blue) with the pattern frequency represented by small horizontal bars with the number involved indicated. (DOCX)

S1 Table. Sensitivity analysis: Examining the effect of selected time period on model results. Time period has been divided into quarters based on PCR testing totals. (DOCX)

S2 Table. Sensitivity analysis: Examining the effect of covariate selection on study findings. (DOCX)

S3 Table. Sensitivity analysis: Examining the effect of covariate selection on study findings. (DOCX)

Author Contributions

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Formal analysis: Andrew Johnston, James Szymanski.

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Methodology: Jui Choudhuri, Jamal Carter, Randin Nelson, Doctor Goldstein, Monika Paroder, James Szymanski.

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Resources: Karin Skalina, Marika Osterbur-Badhey, James Szymanski.

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Supervision: James Szymanski.

Validation: Jamal Carter, James Szymanski.

Visualization: Jui Choudhuri, James Szymanski.

Writing – original draft: Jui Choudhuri, Randin Nelson, Monika Paroder, James Szymanski.

Writing – review & editing: Jui Choudhuri, Jamal Carter, Randin Nelson, Monika Paroder, James Szymanski.

References

1. Yuen KS, Ye ZW, Fung SY, Chan CP, Jin DY. SARS-CoV-2 and COVID-19: The most important research questions. *Cell Biosci.* 2020; 10:40. <https://doi.org/10.1186/s13578-020-00404-4> PMID: 32190290
2. Organization WH. 2020 <https://covid19.who.int/>.
3. Al-Tawfiq JA, Leonardi R, Fasoli G, Rigamonti D. Prevalence and fatality rates of COVID-19: What are the reasons for the wide variations worldwide? *Travel Med Infect Dis.* 2020; 35:101711. <https://doi.org/10.1016/j.tmaid.2020.101711> PMID: 32360326

4. Chang MC, Hur J, Park D. Interpreting the COVID-19 Test Results: A Guide for Physiatrists. *Am J Phys Med Rehabil.* 2020; 99(7):583–5. <https://doi.org/10.1097/PHM.0000000000001471> PMID: 32427600
5. Deming ME, Chen WH. COVID-19 and Lessons to Be Learned from Prior Coronavirus Outbreaks. *Ann Am Thorac Soc.* 2020; 17(7):790–4. <https://doi.org/10.1513/AnnalsATS.202002-149PS> PMID: 32267164
6. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA.* 2020.
7. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical Characteristics of Covid-19 in New York City. *N Engl J Med.* 2020; 382(24):2372–4. <https://doi.org/10.1056/NEJMc2010419> PMID: 32302078
8. Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet.* 2020; 395(10239):1763–70. [https://doi.org/10.1016/S0140-6736\(20\)31189-2](https://doi.org/10.1016/S0140-6736(20)31189-2) PMID: 32442528
9. Argenziano MG, Bruce SL, Slater CL, Tiao JR, Baldwin MR, Barr RG, et al. Characterization and clinical course of 1000 patients with coronavirus disease 2019 in New York: retrospective case series. *BMJ.* 2020; 369:m1996. <https://doi.org/10.1136/bmj.m1996> PMID: 32471884
10. Magleby R, Westblade LF, Trzebucki A, Simon MS, Rajan M, Park J, 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. <https://doi.org/10.1093/cid/ciaa851> PMID: 32603425
11. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther.* 2020; 5(1):33. <https://doi.org/10.1038/s41392-020-0148-4> PMID: 32296069
12. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19—A systematic review. *Life Sci.* 2020; 254:117788. <https://doi.org/10.1016/j.lfs.2020.117788> PMID: 32475810
13. Yu B, Li X, Chen J, Ouyang M, Zhang H, Zhao X, et al. Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *J Thromb Thrombolysis.* 2020. <https://doi.org/10.1007/s11239-020-02171-y> PMID: 32524516
14. Schiffrin EL, Flack JM, Ito S, Muntner P, Webb RC. Hypertension and COVID-19. *Am J Hypertens.* 2020; 33(5):373–4. <https://doi.org/10.1093/ajh/hpaa057> PMID: 32251498
15. Hussain A, Bhowmik B, do Vale Moreira NC. COVID-19 and diabetes: Knowledge in progress. *Diabetes Res Clin Pract.* 2020; 162:108142. <https://doi.org/10.1016/j.diabres.2020.108142> PMID: 32278764
16. Singh AK, Gupta R, Ghosh A, Misra A. Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab Syndr.* 2020; 14(4):303–10. <https://doi.org/10.1016/j.dsx.2020.04.004> PMID: 32298981
17. Rao SN, Manissero D, Steele VR, Pareja J. A Systematic Review of the Clinical Utility of Cycle Threshold Values in the Context of COVID-19. *Infect Dis Ther.* 2020; 9(3):573–86. <https://doi.org/10.1007/s40121-020-00324-3> PMID: 32725536
18. Kawase J, Asakura H, Kurosaki M, Oshiro H, Etoh Y, Ikeda T, et al. Rapid and Accurate Diagnosis Based on Real-Time PCR Cycle Threshold Value for the Identification of *Campylobacter jejuni*, *astA* Gene-Positive *Escherichia coli*, and *eae* Gene-Positive *E. coli*. *Jpn J Infect Dis.* 2018; 71(1):79–84. <https://doi.org/10.7883/yoken.JJID.2017.151> PMID: 29093317
19. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. *Clin Infect Dis.* 2020. <https://doi.org/10.1093/cid/ciaa619> PMID: 32435816
20. Feikin DR, Fu W, Park DE, Shi Q, Higdon MM, Baggett HC, et al. Is Higher Viral Load in the Upper Respiratory Tract Associated With Severe Pneumonia? Findings From the PERCH Study. *Clin Infect Dis.* 2017; 64(suppl_3):S337–S46. <https://doi.org/10.1093/cid/cix148> PMID: 28575373
21. Argyropoulos KV, Serrano A, Hu J, Black M, Feng X, Shen G, 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.
22. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspectives. *Int J Antimicrob Agents.* 2020; 55(5):105951. <https://doi.org/10.1016/j.ijantimicag.2020.105951> PMID: 32234466
23. van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. *J Clin Virol.* 2020; 128:104412. <https://doi.org/10.1016/j.jcv.2020.104412> PMID: 32416600
24. Fung B, Gopez A, Servellita V, Arevalo S, Ho C, Deucher A, et al. Direct Comparison of SARS-CoV-2 Analytical Limits of Detection across Seven Molecular Assays. *J Clin Microbiol.* 2020; 58(9). <https://doi.org/10.1128/JCM.01535-20> PMID: 32651238

25. Lieberman JA, Pepper G, Naccache SN, Huang ML, Jerome KR, Greninger AL. Comparison of Commercially Available and Laboratory-Developed Assays for. *J Clin Microbiol*. 2020; 58(8).
26. Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med* 2020. 2020; S2213-2600(20):30354–4 [https://doi.org/10.1016/S2213-2600\(20\)30354-4](https://doi.org/10.1016/S2213-2600(20)30354-4) PMID: 32771081
27. Aquino-Jarquin G. The raw Ct values from RT-PCR detection are not viral load quantitation units. *Clin Infect Dis*. 2020.
28. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020; 20(5):565–74. [https://doi.org/10.1016/S1473-3099\(20\)30196-1](https://doi.org/10.1016/S1473-3099(20)30196-1) PMID: 32213337
29. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. *N Engl J Med*. 2020; 382(12):1177–9. <https://doi.org/10.1056/NEJMc2001737> PMID: 32074444
30. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, 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> PMID: 32317267
31. 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. <https://doi.org/10.1038/s41591-020-0869-5> PMID: 32296168
32. Huang JT, Ran RX, Lv ZH, Feng LN, Ran CY, Tong YQ, et al. Chronological Changes of Viral Shedding in Adult Inpatients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020. <https://doi.org/10.1093/cid/ciaa631> PMID: 32445580
33. Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020; 63(3):364–74. <https://doi.org/10.1007/s11427-020-1643-8> PMID: 32048163
34. Chen J, Qi T, Liu L, Ling Y, Qian Z, Li T, et al. Clinical progression of patients with COVID-19 in Shanghai, China. *J Infect*. 2020; 80(5):e1–e6. <https://doi.org/10.1016/j.jinf.2020.03.004> PMID: 32171869
35. Bustin SA, Mueller R. Real-time reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clin Sci (Lond)*. 2005; 109(4):365–79.