Association Between Reverse Transcription Polymerase Chain Reaction Cycle Thresholds and Outcomes of Individuals Hospitalized for Coronavirus Disease 2019

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ABSTRACT **Background:** Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to a wide spectrum of clinical severity. The gold standard diagnosis of infection is reverse transcription polymerase chain reaction of nasopharyngeal swabs, which also provides a semiquantitative assessment of viral loads by measuring cycle threshold (CT) values.

Objective: To assess whether CT values at admission can predict mortality and oxygen needs among individuals hospitalized for coronavirus disease 2019 (COVID-19).

Methods: The retrospective study included adults hospitalized for COVID-19 between 1 August 2020 and 30 April 2021 at Barzilai University Medical Center. Patients were categorized according to initial CT values as high (≥ 25) or low (< 25) values. The primary outcome was the association between CT values during admission and overall mortality.

Results: The study group included 636 patients, with a mean age of 67.2 years, 54.4% males. Overall mortality of patients with CT values < 25 was significantly higher (odds ratio for mortality 1.78 vs. patients with CT ≥ 25, $P = 0.002$). Significantly more patients in the low CT group required oxygen support than in the high CT group, 50% vs. 31.9% ($P < 0.001$). An inverse association between CT values and mortality rates remained significant in multivariate regression analysis, such that a 1-unit decrease in CT was associated with a 6% increased mortality.

Conclusions: Lower CT values at admission were associated with increased mortality among patients hospitalized for COVID-19. CT values can be used to predict outcomes among such patients.

IMAJ 2025; 27: 795–799

KEY WORDS: coronavirus disease 2019 (COVID-19), cycle threshold (CT), mortality prediction, polymerase chain reaction (PCR), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can result in diverse clinical presentations, ranging from asymptomatic infection to critical, life-threatening disease with severe lung injury, and multi-organ failure [1]. Early and accurate prediction of disease course may affect management, including identifying needs for hospital admission and monitoring, providing specific treatments, and improving health systems organization. However, currently, prognostication is not precise for individual patients.

Diagnosis of SARS-CoV-2 infection is possible by either identifying viral RNA (polymerase chain reaction [PCR] assays) or viral peptides (antigen assays) in nasopharyngeal swabs. Real-time PCR assays utilize consecutive amplification cycles of fluorescently marked genetic sequences [2]. When the level of fluorescence crosses the detection threshold (cycle threshold [CT]) the assay is considered positive. In addition to high accuracy, such assays can also provide a quantitative estimation of the amount of viral genetic material in the specimen. The assay sensitivity is dependent on the tissue viral load: as the viral load increases so does the amount of target sequences and the tests sensitivity. There is an inverse association between the quantity of RNA sequences in a specimen and the detection threshold: as the amount of specific viral genetic sequences increases, its CT decreases. Thus, CT may serve as an indirect marker of viral load in the sampled tissue, decreasing as the viral load is higher [3,4].

We assessed the association between CT values and outcomes of patients hospitalized for coronavirus disease 2019 (COVID-19). We hypothesized that lower CT values, which reflect higher viral loads, would be associated with worse outcomes.

PATIENTS AND METHODS

We conducted an observational retrospective study. Results are presented according to STROBE guidelines.

STUDY POPULATION

The study population included adult patients (aged > 18 years) who were hospitalized for COVID-19 at Barzilai University Medical Center between 1 August 2020 and 30 April 2021. We included only patients who had undergone a nasopharyngeal swab for SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) in the emergency department. Participants were categorized into one of two groups according to CT values at admission: high CT ≥ 25 and low < 25.

CT MEASUREMENTS

All RT-PCR studies were performed at the medical center's microbiological laboratory using the Real-Time Fluorescent RT-PCR kit for Detecting SARS-CoV-2 (BGI, MA, USA) on a Lightcycler 480 system (Roche Diagnostics, Basel, Switzerland). The assay measures the ORF1ab gene and reports CT accordingly.

DATA COLLECTION AND OUTCOME MEASURES

Data were collected from the medical records and included sociodemographic information, clinical findings, treatment provided, admission length, and vital status at hospital discharge. Laboratory tests results on the day of admission were also collected.

Our primary aim was to assess an association between CT on the day of admission (taken in the emergency department) and overall mortality during admission. Secondary aims included assessing whether there was an association between CT and the need for oxygen support during admission. We measured the association between patient age and sex and CT values at admission. We also evaluated CT values and inflammatory markers at admission.

STATISTICAL METHODS

Descriptive statistics are presented as means ± standard deviations, as medians and interquartile range, or as proportions. Comparison between study groups (CT ≥ 25 / CT < 25) and categorical variables were performed using the chi-square test and Fisher's exact test as appropriate. The variances of quantitative variables of two study groups were compared using ANOVA or Student's *t*-test. The correlation between quantitative variables was calculated with Spearman or Pearson coefficients. A multivariate linear regression analysis was conducted to control for possible confounders affecting an association between CT and mortality. A *P*-value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 24 (SPSS, IBM Corp, Armonk, NY, USA) and STATA v. 9 (StataCorp LLC, College Station, TX, USA).

ETHICS CONSIDERATIONS

The study was approved by the ethics committee and institutional review board of Barzilai University Medical Center (application no. BRZ-0057-21). The committee waived the requirement for participant informed consent due to the retrospective nature of the study. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Table 1. Background characteristics and laboratory findings at admission (346 males [54.4%], 290 females [45.6%])

Variable	Range	Mean ± standard deviation	Median
Age, year	19.0–102.0	67.2 ± 18.9	69.0
Body mass index	13.8–50.8	28.5 ± 5.8	27.8
Creatinine, mg/dl	0.29–10.5	1.40 ± 1.4	1.0
C-reactive protein, mg/L	0.7–406.0	78.8 ± 80.0	51.9
D-dimer, ng/ml	190–122,200	3,067 ± 8,802	1022.0
Blood eosinophils, K/µl	0.0–1.6	0.05 ± 0.1	0.01
Interleukin-6, pg/ml	2.0–4593.0	84.0± 271.0	30.0
Lactate dehydrogenase, U/L	183.0–1964.0	534.0 ± 490.1	461.0
Troponin, ng/L	14.0–1336.0	77.5 ± 128.0	37.0
Leucocytes, × 10^9/L	7.6–137.2	7.6 ± 7.5	6.4
Lymphocytes, × 10^9/L	0.1–112.8	1.6 ± 6.1	1.0
Hemoglobin, g/dl	12.1–23.0	12.1 ± 3.7	12.5
Ferritin, ng/ml	9.8–7427.0	701 ± 886	414

RESULTS

There were 636 patients who were hospitalized for COVID-19 during the study period. All patients had undergone RT-PCR testing from nasopharyngeal swabs in the emergency department on the day of admission. Mean age was 67.2 ± 18.9 years, 346 males (54.4%) and 290 females (45.6%). The background characteristics and laboratory values of the study group at admission are presented in Table 1.

There were 229 individuals with low CT values < 25 (36%). The distribution of CT values is presented in Figure 1.

Patients with low CT values were on average older than those with high CT values, mean age 70.7 ± 18.8 vs. 64.7 ± 18.8 ($P < 0.001$) [Figure 2], yet sex distribution was similar between groups.

CORRELATION BETWEEN CT VALUES AND OUTCOMES

Overall, in-hospital mortality rate was 22.9% (146 of 636). Mortality rate of patients with CT values < 25 was significantly higher, odds ratio for a fatal outcome was 1.78, 95% confidence interval 1.22–2.59, $P = 0.002$. Other variables that were associated with increased mortality were increased age, creatinine, interleukin 6 (IL-6), and C-reactive protein (CRP).

Figure 1. Distribution of cycle threshold values

Mean CT for the study population (n=636) was 26.6 ± 6.7

CT = cycle threshold

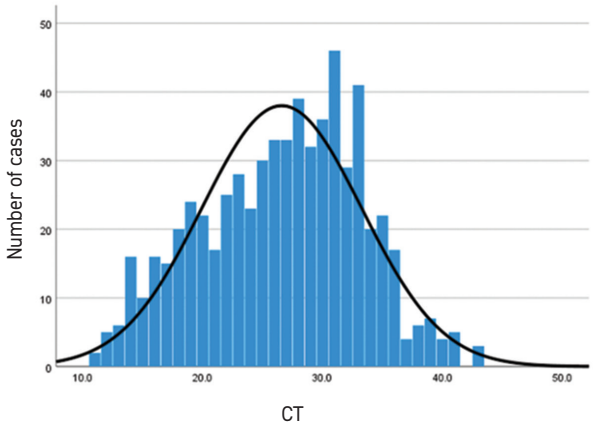
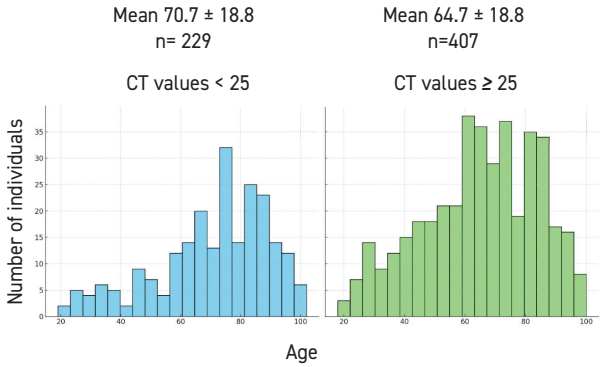


Figure 2. Age distribution according to cycle threshold categories

CT = cycle threshold



In a multivariate linear regression model that incorporated those variables, the inverse association between CT values and mortality rates remained significant, such that a decrease of CT by 1 unit was associated with a 6% increase in mortality [Table 2].

Likewise, more patients in the low CT group required oxygen support during hospitalization: 50% vs. 31.9% in the high CT group ($P < 0.001$).

Regarding medications provided during admission, corticosteroids were administered to 155 patients (24.4%), convalescent plasma to 214 (33.6%), remdesivir to 68 (10.7%), and tocilizumab to 16 (2.5%). In total, 476 patients (74.8%) received antibiotics during their admission. There were no associations between treatment and disease outcomes or between the therapies and CT values.

Table 2. Multivariant regression model for significant predictors of mortality

Variable	OR	95%CI	*P*-value
CT, units	0.942	0.902–0.984	0.007
Age, years	1.079	1.055–1.105	< 0.001
Creatinine, mg/dl	1.422	1.188–1.701	< 0.001
Interleukin-6, pg/ml	1.005	1.002–1.009	0.002
CRP, mg/L	1.004	1.001–1.008	0.012

All variables are assessed as continuum, and according to values at hospital admission.

CRP = C-reactive protein, CT = cycle threshold, OR = odds ratio, 95%CI = 95% confidence interval

We found no association between CT group and laboratory tests results at admission, including blood count, blood chemistry (creatinine, albumin, lactate dehydrogenase), inflammatory markers (CRP, IL-6, ferritin), or D-dimer.

DISCUSSION

The main finding of this study is a significant inverse association between low and high CT groups at admission and mortality during hospitalization for COVID-19.

SARS-CoV-2 infection can lead to highly variable clinical presentations, challenging clinicians to correctly predict disease course early. Accurate diagnosis can aid in clinical decisions such as deciding on hospitalization, additional testing or monitoring, and specific therapies. Proper diagnosis ensures that medical resources are properly allocated. COVID-19 outcomes are dependent

on several elements of the infection and host. Studies have generated prognostic scoring systems that incorporate several variables [5]. Some variables that increase the risk for critical disease and mortality are related to the patient, including increased age, male sex, and obesity. Many co-morbidities, such as diabetes mellitus, hypertension, cardiovascular diseases, chronic kidney disease, active malignancies, and immunosuppression are also associated with increased risk and may help predict outcomes [6,7]. Several clinical variables correlate with prognosis, especially vital signs (e.g., oxygen saturation, blood pressure, heart and respiratory rates) and laboratory findings (e.g., white blood cells count, neutrophil-to-lymphocyte ratio, serum creatinine, albumin, CRP, D-dimer, and IL-6) [8-10]. Factors associated with the pathogen include different variants of the virus. Infections with the Delta variant (B.1.617.2) more commonly led to severe COVID-19 and increased risk for hospitalization and mortality, while the Omicron variant was highly transmittable even among vaccinated populations, yet usually caused mild disease [11,12]. Vaccination against SARS-CoV-2, as well as prior infection, generally protect against severe COVID-19 and mortality [13]. Thus, breakthrough infections of vaccinated individuals usually result in mild disease. Despite accumulated knowledge regarding COVID-19 risk factors, individual prognostication is still challenging and imprecise.

When infection is diagnosed by RT-PCR, the assays may also provide semi-quantitative assessment of viral load. There is an inverse correlation between CT and viral load, each 3.3 units decrease in CT are associated with a 10-fold increase in viral load [14]. The association between viral loads, as reflected in CT values, and COVID-19 outcomes has been evaluated in several studies with equivocal results. Some studies have demonstrated an inverse correlation between parameters of COVID-19 severity and mortality and between CT values, both among general population and specific groups such as cancer patients [15,16]. In other studies, such correlation was excluded, either during multivariant analysis or during the original analysis [17]. A systematic review could not identify a definitive association between CT values and COVID-19 severity [18]. However, assessing the association between viral load/CT values and outcomes may be affected by several factors.

We found that CT was associated with age, such that CT values were lower among older patients. This finding is comparable to prior publications [19]. Age is a significant risk factor for COVID-19 outcomes, with worse prognosis and increased mortality among older patients. Thus, it is possible that age is a confounder of results, associated both with worse outcomes and lower CT values. However, the correlation between CT and mortality remained significant even when other variables were controlled by multivariate analysis [Table 2]. Similar results were observed in prior studies. No association was identified between co-morbidities and viral load in previous studies other than a positive correlation between immunosuppressive therapy for hematological malignancies and increased viral load [20].

CT values vary during disease course: low values were noted during the first days of COVID-19 symptoms and gradually increased thereafter [14]. Lack of association between CT values and outcomes may result from inconsistent timing of PCR relative to disease onset. While we tried to overcome this issue by uniformly including patients who had been tested when they arrived at the emergency department, we have no data regarding symptoms duration prior to admission or time of infection.

We assessed the association between CT values, mortality, and oxygen therapy since those outcomes were less confounded by factors unrelated to disease severity. Decisions regarding hospital admission are impacted by social status and isolation requirements. Local availability and an individual's baseline status impact on admission to intensive care units, such that very ill patients with slim chances of recovery, as well as patients presenting with advanced chronic diseases or dementia, are usually not admitted. Management and healthcare resources also differed between different countries and geographical location. We chose overall mortality as the primary outcome to overcome such confounders.

Several technical issues can also affect CT values. Concerns include utilizing different assays for RT-PCR in different laboratories, inconsistencies in sample storage duration, handling and transportation prior to testing, and type of samples collected (e.g., nasopharyngeal swabs, saliva, or lower respiratory tract samples) [19,20]. In our study, all analyses were performed from nasopharyngeal swabs with the same assay on the day of collection and in the same in-center laboratory without need for prolonged storage or transportation. This consistency may have reduced the possible impact of technical variables on CT results.

It should be noted that a publication bias may exist, in which studies that did not find an association between CT and COVID-19 outcomes were not published.

Our study has several limitations. The retrospective, single-center design of the study may have led to biased results. Co-morbidities, which are major determinants of COVID-19 outcome, were not assessed or included in the

multivariant analysis. Age was a significant predictor of both CT values (inverse correlation) and mortality. We tried to control its confounding effects by multivariate regression model analysis. Data regarding drug therapy for COVID-19 have evolved since the time of this study, with validated indications for the use of remdesivir, corticosteroids, and tocilizumab. Convalescent plasma has not demonstrated benefit, and antibiotics are required only for selected patients with high suspicions or evidence for concomitant bacterial infections. In addition, the study period was prior to the emergence of clinically relevant SARS-CoV-2 variants in Israel and before the availability of vaccines. Thus, the results are not necessarily generalizable to a vaccinated population with viral variants under current therapeutic approaches. However, our results may reflect the natural course of infection with the wild type virus.

CONCLUSIONS

We demonstrated an inverse association between CT values at hospital admission and oxygen requirements and mortality from SARS-CoV-2 infection. Despite the limitations, consistency of the results suggests that CT is a reliable predictor of severity and mortality from COVID-19. Our results support the inclusion of CT in individual risk stratification models among patients with SARS-CoV-2 infection, together with other sociodemographic and clinical parameters.

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