



Original Article

Association of RT-PCR Cycle Threshold Values with Disease Severity and Clinical Outcomes in Hospitalized COVID-19 Patients: A Prospective Observational Study

Dr. Rashmi¹, Dr. Ankit Garg², Dr. Sushila Kataria³, Dr. Vikas Deswal⁴

¹Assistant Professor, Deptt. of Medical Microbiology, FMHS, SGT University, Budhera, Gurugram.

²Senior resident, Deptt. of Internal Medicine, FMHS, SGT University, Budhera, Gurugram.

³Senior director, Deptt of Internal Medicine, Medanta- The Medicity, Gurugram.

⁴Senior Consultant, Deptt of Internal Medicine, Medanta- The Medicity, Gurugram

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Corresponding Author:

Dr. Rashmi

Assistant Professor Deptt. of
Medical Microbiology, FMHS,
SGT University, Budhera,
Gurugram.

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ABSTRACT

Background: RT-PCR cycle threshold (Ct) values are commonly used as a surrogate of SARS-CoV-2 viral burden, but their prognostic utility and dependence on gene targets remain uncertain. We evaluated the association of gene-specific Ct values with disease severity and clinical outcomes among hospitalized COVID-19 patients.

Methods: This prospective observational study included 447 adults with RT-PCR-confirmed COVID-19 admitted to a tertiary care centre in North India. Admission Ct values for the N gene and RdRP gene were analysed across severity categories and by ICU admission, invasive mechanical ventilation, and in-hospital mortality.

Results: Ct values declined with increasing severity. N-gene Ct values differed significantly across mild, moderate, and severe disease ($p = 0.024$), while the corresponding trend for RdRP was not statistically significant ($p = 0.274$). Patients requiring ICU care had significantly lower Ct values than those not requiring ICU admission (N gene $p < 0.0001$; RdRP $p = 0.001$). Similarly, patients requiring invasive mechanical ventilation had lower Ct values for both targets ($p < 0.0001$ for each). Although non-survivors had numerically lower Ct values than survivors, the differences were not statistically significant for either gene.

Conclusions: Lower admission Ct values were associated with greater disease severity and need for advanced respiratory support, with stronger prognostic discrimination for the N gene than the RdRP gene. Ct values may aid early risk stratification when interpreted alongside clinical parameters, but should not be used as standalone predictors of mortality.

Keywords: COVID-19, RT-PCR Cycle Threshold (Ct) Value, Viral Load, Disease Severity, Mechanical Ventilation.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is characterized by marked clinical heterogeneity, with a subset of hospitalized patients progressing rapidly to severe illness requiring intensive care and advanced respiratory support [1,2]. Early identification of patients at risk for adverse outcomes remains essential for timely triage and optimal resource utilization. Although several predictors of COVID-19 severity have been described, including age, comorbidities, and inflammatory biomarkers, many of these parameters become abnormal only after disease progression, limiting their value for early risk stratification [3,4]. This has prompted interest in prognostic markers that are readily available at the time of diagnosis.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR), the diagnostic gold standard for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), provides a cycle threshold (Ct) value that is inversely related to viral RNA burden [5]. While Ct values are routinely reported, their prognostic relevance remains uncertain because of inter-assay variability and lack of standardized interpretation [5,6]. Several studies have demonstrated that higher SARS-CoV-2

viral load, reflected by lower RT-PCR cycle threshold values, is associated with increased disease severity and adverse clinical outcomes in hospitalized patients [7–9]. In contrast, other studies have reported no consistent relationship, highlighting ongoing controversy [10,11].

Importantly, Ct values may vary depending on the viral gene target used for RT-PCR amplification, most commonly the nucleocapsid (N) and RNA-dependent RNA polymerase (RdRP) genes [12,13]. Limited data exist comparing the prognostic significance of gene-specific Ct values, particularly in hospitalized patients from low- and middle-income countries [14,15].

This prospective observational study therefore aimed to evaluate the association between RT-PCR Ct values and disease severity and clinical outcomes in hospitalized COVID-19 patients, with a specific focus on comparing N and RdRP gene targets.

MATERIALS AND METHODS

Study design and setting

This prospective observational cohort study was conducted at Medanta – The Medicity, Gurugram, a tertiary care referral center in North India, during a four-month period of the COVID-19 pandemic. The study protocol was reviewed and approved by the Institutional Ethics Committee of Medanta – The Medicity, Gurugram (Approval No.: MICR-1186/2020). Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment. Consecutive adult patients admitted with confirmed coronavirus disease 2019 (COVID-19) were enrolled to minimize selection bias.

Study population

Adult patients aged 18 years or older with laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection were screened for inclusion. Only patients who underwent RT-PCR testing using the institutional assay within seven days of symptom onset were included to ensure uniformity of viral load assessment. Patients tested using non-institutional RT-PCR kits, those presenting beyond seven days of symptom onset, and patients referred after initial diagnosis elsewhere were excluded. Cases with cycle threshold (Ct) values greater than 30 were excluded from quantitative viral load analysis.

Clinical assessment and severity classification

Baseline demographic characteristics, comorbidities, and clinical parameters were recorded at admission using a standardized data collection form. Disease severity was categorized at presentation into mild, moderate, or severe illness in accordance with institutional and national guidelines. Severity classification was based on clinical features, respiratory rate, peripheral oxygen saturation on room air, and the requirement for supplemental oxygen, non-invasive ventilation, or invasive mechanical ventilation.

RT-PCR testing and cycle threshold value analysis

Nasopharyngeal swab specimens were collected at admission by trained health-care personnel following standard biosafety protocols. Samples were processed in the institutional molecular diagnostics laboratory using the government-approved Labgun COVID-19 ExoFast RT-PCR kit, targeting the nucleocapsid (N) gene and the RNA-dependent RNA polymerase (RdRP) gene. Ct values corresponding to both gene targets were recorded for each patient. All samples were processed using the same platform and protocol to minimize inter-assay variability. Lower Ct values were interpreted as reflecting higher viral RNA burden.

Outcome measures

The primary outcome of interest was disease severity at hospital admission. Secondary outcomes included requirement for intensive care unit (ICU) admission, need for invasive mechanical ventilation, and in-hospital mortality. Patients were followed prospectively until discharge or death.

Sample size estimation

Sample size estimation was performed to detect a clinically meaningful difference in mean Ct values between patients with mild-to-moderate disease and those with severe disease. Assuming a mean difference of approximately three Ct cycles, a standard deviation of 12–14 cycles, a two-sided alpha of 0.05, and 90% power, the minimum required sample size was calculated as 411 patients. A total of 447 patients were ultimately included in the analysis.

Statistical analysis

Continuous variables were expressed as mean \pm standard deviation or median with interquartile range, as appropriate. Categorical variables were summarized as frequencies and percentages. Differences in mean Ct values across severity categories were assessed using one-way analysis of variance (ANOVA), while comparisons between two groups were performed using Student's t-test. A two-sided p value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS software.

RESULTS

Baseline characteristics

A total of 447 hospitalized patients with confirmed COVID-19 were included in the study. Baseline demographic and clinical characteristics are summarized in **Table 1**. The mean age of the cohort was 59.2 ± 14.0 years. Diabetes mellitus was the most common comorbidity, followed by coronary artery disease, chronic kidney disease, malignancy, chronic obstructive pulmonary disease, and chronic liver disease.

Table 1. Baseline demographic and clinical characteristics of hospitalized COVID-19 patients (n = 447)

Characteristic	Value
Age (years), mean \pm SD	59.2 ± 14.0
Comorbidities, n (%)	
Diabetes mellitus (DM)	159 (36.1%)
Coronary artery disease (CAD)	65 (14.5%)
Chronic kidney disease (CKD)	41 (9.2%)
Chronic obstructive pulmonary disease (COPD)	30 (6.7%)
Chronic liver disease (CLD)	15 (3.4%)
Malignancy (CA)	37 (8.3%)

*Data are presented as mean \pm standard deviation or number (percentage), as appropriate.

Table 2. Relationship between RT-PCR cycle threshold (Ct) values and disease severity.

Disease severity	N gene Ct (mean \pm SD)	RdRP gene Ct (mean \pm SD)
Mild	20.0 ± 5.4	20.5 ± 5.6
Moderate	18.4 ± 5.3	19.7 ± 6.3
Severe	17.9 ± 6.7	19.1 ± 6.7
P value (ANOVA)	0.024	0.274

*Data are expressed as mean \pm standard deviation. *P* values were calculated using one-way analysis of variance (ANOVA).

Table 3. Association of N gene cycle threshold (Ct) values with ICU admission, intubation, and mortality

Clinical outcome	Group	n	N gene Ct (mean \pm SD)	Mean difference	P value
ICU admission	Yes	73	16.3 ± 6.5	-2.8	<0.0001*
	No	371	19.1 ± 5.5		
Intubation	Yes	61	16.2 ± 5.7	-2.8	<0.0001*
	No	365	19.5 ± 5.7		
Mortality	Yes	24	16.6 ± 6.1	-2.1	0.076
	No	422	18.8 ± 5.7		

*Data are expressed as mean \pm standard deviation. *P* values were calculated using Student's *t*-test. *P* < 0.05 was considered statistically significant.

Ct values and disease severity

Mean RT-PCR Ct values demonstrated a stepwise decline with increasing disease severity, most prominently for the N gene. The mean N-gene Ct value decreased from 20.0 ± 5.4 in patients with mild disease to 18.4 ± 5.3 in those with moderate disease and 17.9 ± 6.7 in patients with severe disease. This inverse association between N-gene Ct values and disease severity was statistically significant (*p* = 0.024).

For the RdRP gene, Ct values also showed a downward trend across severity categories (20.5 ± 5.6 in mild, 19.7 ± 6.3 in moderate, and 19.1 ± 6.7 in severe disease); however, this association did not reach statistical significance (*p* = 0.274) (Table 2).

Ct values and clinical outcomes

The association of N-gene Ct values with ICU admission, intubation, and mortality is presented in Table 3. Patients requiring ICU admission had significantly lower N-gene Ct values compared with non-ICU patients (16.3 ± 6.5 vs 19.1 ± 5.5 ; mean difference -2.8; *p* < 0.0001). Similarly, patients requiring invasive mechanical ventilation demonstrated significantly lower N-gene Ct values than non-intubated patients (16.2 ± 5.7 vs 19.5 ± 5.7 ; mean difference -2.8; *p* < 0.0001). Although non-survivors showed numerically lower N-gene Ct values compared with survivors (16.6 ± 6.1 vs 18.8 ± 5.7 ; mean difference -2.1), the association was not statistically significant (*p* = 0.076).

The association of RdRP-gene Ct values with clinical outcomes is shown in **Table 4**. Patients admitted to the ICU had significantly lower RdRP-gene Ct values compared with non-ICU patients (17.5 ± 6.5 vs 20.2 ± 6.1 ; mean difference -2.7; *p* = 0.001). Likewise, intubated patients demonstrated significantly lower RdRP-gene Ct values than non-intubated patients (17.0 ± 5.9 vs 20.2 ± 6.3 ; mean difference -3.2; *p* < 0.0001). Non-survivors had lower RdRP-gene Ct values compared

with survivors (17.8 ± 6.7 vs 19.9 ± 6.2 ; mean difference -2.1), although this difference was not statistically significant ($p = 0.110$).

Table 4. Association of RdRP gene cycle threshold (Ct) values with ICU admission, intubation, and mortality

Clinical outcome	Group	n	RdRP gene Ct (mean \pm SD)	Mean difference	P value
ICU admission	Yes	73	17.5 ± 6.5	-2.7	0.001*
	No	371	20.2 ± 6.1		
Intubation	Yes	61	17.0 ± 5.9	-3.2	<0.0001*
	No	365	20.2 ± 6.3		
Mortality	Yes	24	17.8 ± 6.7	-2.1	0.110
	No	422	19.9 ± 6.2		

*Data are expressed as mean \pm standard deviation. *P* values were calculated using Student's *t*-test. $P < 0.05$ was considered statistically significant.

DISCUSSION

In this prospective observational study of hospitalized patients with COVID-19, lower RT-PCR cycle threshold values were associated with greater disease severity, most notably for the N gene. The study cohort comprised predominantly older adults with a high burden of comorbidities, reflecting the typical profile of patients requiring hospital admission. Within this context, N-gene Ct values demonstrated a significant stepwise decline from mild to severe disease, whereas the corresponding trend for RdRP gene Ct values did not reach statistical significance.

These findings are consistent with prior reports demonstrating an association between higher viral RNA burden and adverse clinical outcomes. Magleby et al. (2021) and Pujadas et al. (2020) reported that higher viral load at presentation was associated with increased risk of clinical deterioration and need for advanced respiratory support [16,17]. Similarly, Zacharioudakis et al. (2021) and Al Bayat et al. (2021) observed lower Ct values among patients with severe disease and those requiring intensive care, supporting the prognostic relevance of Ct values in hospitalized cohorts [18,19].

An important observation of the present study is the differential performance of gene targets. Ct values are known to be assay- and gene-dependent. Dahdouh et al. (2021) demonstrated that nucleocapsid gene targets often exhibit greater analytical sensitivity compared with other viral genes, which may account for the stronger association observed for N-gene Ct values in our cohort [20]. Singanayagam et al. (2020) further showed that lower Ct values correlate with higher viral infectivity, providing biological plausibility for the observed relationship between N-gene Ct values and disease severity [21].

Lower Ct values were also significantly associated with intensive care unit admission and need for invasive mechanical ventilation. Miller et al. (2022) reported similar associations between higher viral load and respiratory failure, while Patterson et al. (2020) described viral-load-driven immune dysregulation contributing to severe disease, supporting the clinical relevance of these findings [22,23]. In contrast, although non-survivors in the present study had numerically lower Ct values than survivors, this difference did not reach statistical significance, suggesting that mortality is influenced by multiple host and treatment-related factors beyond viral burden alone, as previously reported by Zhou et al. (2020) and the RECOVERY Collaborative Group (2021) [24,25].

Data from low- and middle-income countries on Ct value interpretation remain limited. Indian studies have reported heterogeneous associations between viral load and outcomes, reflecting differences in timing of presentation and health-care access [26,27]. Importantly, Ct values are influenced by pre-analytical and assay-related factors and should therefore be interpreted cautiously and in conjunction with clinical parameters rather than as standalone prognostic markers, as emphasized by Rhee et al. (2021), Miller et al. (2021), and Mina et al. (2020) [28–30].

CONCLUSION

Lower RT-PCR cycle threshold values were associated with greater disease severity and need for advanced respiratory support among hospitalized patients with COVID-19, with the N gene demonstrating stronger prognostic utility than the RdRP gene. These findings suggest that gene-specific Ct values, when interpreted in conjunction with clinical parameters, may aid early risk stratification of hospitalized patients. However, Ct values should not be used in isolation, as mortality outcomes are influenced by multiple host and treatment-related factors. Further prospective studies are warranted to standardize the clinical application of Ct values across assays and patient populations.

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