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Association Between SARS-CoV-2 Cycle Threshold Values at Hospital Admission and Clinical Outcomes in COVID-19 Patients: A Retrospective Cohort Study.

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ABSTRACT

Background: Cycle Threshold (Ct) values from SARS-CoV-2 RT-qPCR tests have been widely investigated as prognostic markers. However, the interpretation of Ct values is fundamentally dependent on the time of sampling relative to symptom onset a low value may indicate normal early replication or, conversely, a failure of viral clearance later in the disease course. Many studies fail to account for this critical confounder.

Objective: Using a real-world cohort, we demonstrate how the absence of symptom onset data renders prognostic analysis of Ct values uninterpretable and provide a framework for mandatory reporting.

Methods: We conducted a retrospective analysis of 455 hospitalized COVID-19 patients (04.08.2020–11.01.2022) with admission RT-qPCR results (RdRp and N gene targets). Symptom onset data was unavailable. We performed standard prognostic analyses: Ct values were correlated with clinical outcomes, inflammatory markers (IL-6, D-dimer, CRP), and organ dysfunction. Univariate analysis, ROC (Receiver Operating Characteristic) curve analysis (for mortality), and multivariate regression were conducted as if symptom onset were unknown simulating a common methodological flaw.

Results: In this cohort (61.8% male, mortality 10.2%), lower Ct values (indicating higher viral RNA) showed statistically significant inverse correlations with key severity markers: IL-6 ($r = -0.239$, $p<0.001$), creatinine ($r = -0.235$, $p<0.001$), and need for oxygen ($p<0.001$). ROC analysis for mortality yielded an AUC (Area Under the Curve) of 0.746 with an optimal Ct cut-off value 24.5. A multivariate model identified IL-6, creatinine, and SpO2 as significant correlates of Ct but explained only 14.2% of variance ($R^2=0.142$), suggesting missing critical explanatory variables.

Conclusion: This study replicates common analytical approaches and yields seemingly significant associations between Ct values and disease severity. However, in the definitive absence of symptom onset data, the key to contextualizing viral load and these results are clinically uninterpretable and potentially misleading. We propose that any study investigating Ct values as a prognostic biomarker must explicitly report and adjust for time since symptom onset; failure to do so constitutes a fundamental methodological error. Our findings serve as a critical case study for standardizing future research

Key Words: SARS-CoV-2; COVID-19; RT-qPCR; Cycle Threshold; Prognosis; Research Methodology; Symptom Onset; Confounding Factors.

INTRODUCTION

SARS-COVID-19 is a coronavirus that has continually necessitated a global medical system to approach its comprehensive clinical manifestation, including asymptomatic infection, to terminal multi-organ failure (Wu Z et al., 2020; Lopes-Pacheco et al., 2021). Early detection of high-risk patients in hospital settings who are likely to deteriorate clinically, get admitted to an intensive care unit, and die has been identified as a critical issue in managing the patient and in resource allocation (Angeli et al., 2021). Although the use of clinical risk scores and biomarkers such as D-dimer, CRP and ferritin has been made, there is an urgent need to identify prognostic indicators that are simple,

rapidly available, and objective to aid in making decisions during and before the admission of patients (Bae et al., 2021).

The time dependence of the clinical course of the infection with SARS-CoV-2 is connected with its nature. The incubation period, which is the span between exposure and the onset of symptoms, is between 2 and 14 days in the post-exposure period (Lauer et al., 2020). The first symptoms are most frequently non-specific, as they commonly include fever, cough, fatigue, sore throat, and the typical loss of taste or smell (Guan et al., 2020). More importantly, the course of illness is never consistent. Although most of the patients resolve without any complications, a substantial proportion of patients develop progressive respiratory and systemic inflammation, which is usually demonstrated as an eventual increase in the severity of symptoms within 5 to 7 days of the onset (Zhou et al., 2020). It is a timeline that is now well established to provide a critical context to interpret any snapshot measurement of disease activity, including viral load.

Real-time reverse transcription polymerase chain reaction (RT-PCR) is still the primary diagnostic tool in the case of SARS-CoV-2. In addition to its qualitative outcome, a quantitative Cycle Threshold (Ct) value that is the cycle number to detect the amplification of the viral target signal over background gives a semi-quantitative measure of the viral nucleic acid load in the sample (Buchan et al., 2020). Since the pathophysiological assumption is that an increased viral loading can contribute to more severe disease, Ct values have undergone much research as a possible prognostic measure (Emery et al., 2004). A large literature base of statistically significant relationships exists between the reduced Ct values (reflecting elevated viral RNA) and poor clinical outcome, such as hypoxemia, intensive care unit admission, and mortality (Liu et al., 2020; Platten et al., 2021). Nevertheless, there is a high degree of heterogeneity and contradiction in this body of evidence, many studies do not reveal an independent prognostic value of Ct values when controlled by other factors and this implies that there is contradictory guidance and clinical uncertainty on its use (Rabaan et al., 2020; Rao et al., 2020).

The reason behind this contradiction in the literature is a widely overlooked biological and methodological law: a Ct value is a fixed value of a moving process. The classic study of SARS-CoV-2 viral kinetics shows that the peak of viral loads occurs approximately at the time of symptoms and then it drops, although the direction and speed of this clearance vary significantly between mild and severe patients (Talic et al., 2021). A patient sampled early in their symptomatic period (e.g., day 2) will be in the normal range of viral expansion and will be able to have the same Ct as a patient a week into illness indicating a breakdown of immune-mediated viral clearance and foreshadowing a severe course of illness (Tso et al., 2021). The time that has passed since the onset of symptoms, therefore, confounds the clinical meaning of any given Ct value. Nevertheless, a systematic review of the studies conducted by (Rao et al., 2020) has demonstrated that most early prognostic studies either failed to measure or failed to control this critical time variable and used Ct as a context-free number (Wynants et al., 2020).

Study Rationale and Objective

We posit that the common omission of symptom onset data represents a fundamental methodological flaw that confounds the existing evidence base. To empirically demonstrate this problem, we conducted an analysis using a retrospective cohort of hospitalized COVID-19 patients in which mirroring a common scenario in clinical practice and research the date of symptom onset was not systematically available. Our objective was twofold such as first, to perform the standard suite of prognostic analyses (correlation, regression, ROC analysis) on admission Ct values, thereby replicating the approach of numerous published studies; and second, to use our findings as a case study to illustrate why such analyses, while potentially yielding statistically significant results, are clinically uninterpretable and potentially misleading in the absence of this essential confounder. Through this demonstration, we aim to highlight a pervasive methodological shortcoming and argue for the mandatory reporting and adjustment of time since symptom onset as a non-negotiable standard in all future research evaluating the prognostic utility of SARS-CoV-2 Ct values.

Methods

Study Design and Setting

A retrospective cohort study was conducted at the Jagannath Gupta Institute of Medical Sciences and Hospital, Budge Budge, Kolkata, India. The study period involved patients admitted with a primary diagnosis of COVID-19 between August 4, 2020, and January 11, 2022.

Study Participants

Clinical eligibility was limited to all consecutive adult patients (age ≥18 years) who had been admitted to the hospital with a laboratory confirmed diagnosis of SARS-CoV-2 infection by RT-qPCR from nasopharyngeal/oropharyngeal swab samples. The patients were identified using the electronic medical record (EMR) system of the hospital. The main exclusion criteria were no recorded Cycle Threshold (Ct) value of the admission RT-qPCR test, and missing baseline clinical or laboratory data, which should be used in the analysis.

Critical Data Omission:

They clearly mention that the date that the patient started experiencing the symptoms before presentation to the hospital was not an obligatory field in the standardized admission documentation at the time of the study. Therefore, the exact period between the symptoms onset and the admission RT-qPCR swab (the most important variable of days from symptom onset

to test) was not recorded systematically in the EMR and could not be analyzed in this case, which was the case with the vast majority of patients in this cohort. This is the situation in a typical clinical dataset in the real world, and it is the major hypothesis of this methodological study.

Laboratory Methods

A commercial real-time RT-PCR assay was used to determine SARS-CoV-2 and Ct value. The assay was a concomitant target of two conserved regions of the SARS-CoV-2 genome such as the RNA-dependent RNA polymerase (RdRp) gene and the Nucleocapsid (N) gene. Nasopharyngeal and oropharyngeal swabs were processed with a standardized process of RNA extraction based on silica-membrane. A real-time PCR system (BioRad 96 connect PCR machine) was amplified and detected as per the instructions of the manufacturer. The instrument software automatically plotted the Ct value as the number of cycles when the fluorescence [FAM (6-Carboxyfluorescein) & HEX (Hexachloro-fluorescein)] signal reached above the background threshold. All analyses used the lower of the two values of the target Ct values of the genes (or the one value available in single gene positivity) of the admission test.

Variables and Data Collection

Data were manually abstracted from the hospital's EMR into a structured database. The primary exposure variable was the admission SARS-CoV-2 RT-PCR Ct value, analyzed both as a continuous variable and categorized *post-hoc* into four groups for descriptive purposes: Very High (Ct 31-34), High (Ct 27-30), Intermediate (Ct 23-26), and Low (Ct 12-22).

We collected basic demographics like age and sex to understand the patients group. Vital Signs and Clinical status are Oxygen saturation (SpO2) on room air on admission, and the occurrence of certain symptoms at presentation (e.g. breathlessness, sore throat, loss of taste or smell). Comorbidities: Diagnosed with hypertension, diabetes mellitus, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), or heart failure, and the medical record indicates that the patient has these conditions. Parameters in Laboratory: The closest measurements were taken to the time of admission (white blood cell count (WBC), hemoglobin (Hb), lactate dehydrogenase (LDH), serum ferritin, interleukin-6 (IL-6), serum creatinine, D-dimer, and C-reactive protein (CRP). Radiological Findings: The infiltrates found in the initial chest X-ray were identified as a binary variable (present/absent). Clinical Outcomes: In-hospital mortality was used as the primary outcome in the prognostic analysis. The other outcome measures were the necessity to receive supplemental oxygen at admission.

Ethics approval

The Institutional Ethics Committee of Jagannath Gupta Institute of Medical Sciences and Hospital (JIMSH), Budge Budge, Kolkata was approved this study. (JIMSH-IEC-11-2021 dated 22-11-2021).

Statistical Analysis

The method of analysis aimed to duplicate the methodologies that are frequently used in literature and that aim at determining Ct values as prognostic biomarkers. All of the analyses conducted without time adjustment because we did not have the onset of the symptoms; hence, we simulated the methodology constraint we were testing.

Continuous variables will be provided as mean + standard deviation or median (interquartile range), as necessary. Frequencies and percentages are used to show categorical variables. The associations between categorized Ct values and other clinical variables were done through Chi-square test of categorized variables. Pearson correlation coefficient used to test the strength and direction of linear relationships between the continuous Ct values and other continuous parameters. The Receiver Operating Characteristic (ROC) curve analysis used to assess the discriminatory ability of the admission Ct value to predict in-hospital mortality. The 95% confidence interval under the curve determined and obtained as Area under the Curve (AUC). The Youden index (J = sensitivity + specificity -1) used to determine the most optimum Ct cut-off point (Xu et al., 2014). A multivariate linear regression model developed to determine independent correlates of Ct value and to measure the variance that covered by the available clinical data that were commonly accessible. The dependent variable referred to as the continuous Ct value. All the gathered demographic, clinical, and laboratory variables were effectively inputted into a single step as independent predictors in order to form an all-embracive, yet exploratory, model. The R 2 and adjusted R 2 used to evaluate the model fit. The variance inflation factors (VIF) investigated to test the presence of multicollinearity. All the statistical procedures were conducted with the assistance of IBM SPSS Statistics Windows version
22.0 (IBM Corp., Armonk, NY, USA) and MedCalc Statistical Software version 22.023 (MedCalc Software Ltd, Ostend, Belgium). A p-value of less than 0.05 on both sides was taken as significant at the statistical level.

Results

Out of 656 hospitalized COVID-19 patients, 455 patients met the inclusion criteria and formed the final study population where 201 patients were excluded due to incomplete laboratory or radiological data. *Demographic, clinical, comorbidity, and laboratory characteristics of the cohort are summarized in Table 1.* The mean age of the cohort was 56.5 ± 5.2 years which was predominantly male (281, 61.8%), and had an in-hospital mortality rate of 10.21% (67/656 in the initial screen). Common comorbidities included hypertension (29.2%) and diabetes (20.0%). The mean (at admission period) RT-qPCR Ct

value was 27.1 (±4.2), with a distribution across pre-defined categories: Very High (Ct 31-34, n=117, 25.7%), High (Ct 27-30, n=150, 33.0%), Intermediate (Ct 23-26, n=121, 26.6%), and Low (Ct 12-22, n=67, 14.7%).

Table 1: Baseline Characteristics of the Study Cohort (N=455)

Variable	**Mean ± SD or n (%)**
Demographics	
Age (years)	56.5 ± 5.2
Male sex	281 (61.8%)
Admission Ct value	**27.07 ± 4.20**
Ct category: Low (12–22)	67 (14.7%)
Ct category: Intermediate (23–26)	121 (26.6%)
Ct category: High (27–30)	150 (33.0%)
Ct category: Very High (31–34)	117 (25.7%)
Clinical / HR-CT Findings	
HR-CT / Infection detected in initial chest X-ray	225 (49.5%)
SpO_2 < 94%	72 (15.8%)
Breathlessness	47 (10.3%)
Loss of smell	238 (52.3%)
Loss of taste	251 (55.2%)
Sore throat	85 (18.7%)
Key Comorbidities	
Hypertension	133 (29.2%)
Diabetes mellitus	91 (20.0%)
Chronic kidney disease (CKD)	11 (2.4%)
Chronic obstructive pulmonary disease (COPD)	16 (3.5%)
Heart failure	12 (2.6%)
Selected Admission Laboratory Parameters	
CRP > 6 mg/dL	236 (51.9%)
WBC < 4000/µL	103 (22.6%)
Hemoglobin (Hb) < 9 g/dL	33 (7.3%)
Ferritin > 400 ng/mL	133 (29.2%)
IL-6 > 10 pg/mL	135 (29.7%)
LDH > 400 U/L	350 (76.9%)
Creatinine > 1 mg/dL	79 (17.4%)
D-dimer > 500 ng/mL	149 (32.8%)

Outcome	
In-hospital mortality	10.21% (67/656)

Associations Between Ct Values and Clinical Parameters Without Symptom Onset Data

We analyzed without adjustment for time since symptom onset simulating a common methodological approach lower Ct values (indicating higher viral RNA) showed statistically significant but weak to moderate inverse correlations with key markers of disease severity. As shown in the correlation matrix (**Table 2),** Ct value correlated negatively with interleukin-6 (IL-6, r = -0.239, p<0.001), serum creatinine (r = -0.235, p<0.001), and D-dimer (r = -0.166, p<0.001). Lower Ct categories were also associated with a higher frequency of abnormal chest X-rays (during admission) and a greater need for supplemental oxygen support (**Figure. 1**).

Table 2: Selected Pearson Correlation Coefficients of Ct Value with Clinical Variables

Variable	Correlation with Ct (r)	p-value
Interleukin-6 (IL-6)	-0.239	**<0.001**
Serum Creatinine	-0.235	**<0.001**
D-dimer	-0.166	**<0.001**
SpO_2	0.112	**0.008**
C-reactive Protein (CRP)	0.026	0.289
White Blood Cell Count (WBC)	-0.149	**0.001**

A multivariable linear regression model was constructed with Ct value as the dependent variable and rest all like demographic, clinical, and laboratory parameters as independent predictors (**Table 3)**. This model, representative of analyses attempting to identify factors associated with viral load, was confirmed IL-6 (B = -1.63, p<0.001), creatinine (B = -1.83, p=0.001), low SpO_2 (B = -1.29, p=0.013), and WBC (B = -1.04, p=0.024) as statistically significant independent correlates of a lower Ct value.

Prognostic Performance and Model Limitations

Receiver Operating Characteristic (ROC) curve analysis performed to assess the standalone prognostic value of the admission Ct value for in-hospital mortality which was yielded an area under the curve (AUC) of 0.746 (95% CI: 0.685–0.807, p<0.001). The optimal cut-off of Ct value was identified at 24.5, providing a sensitivity of 73.8% and a specificity of 88.8% **(Figure. 2).**

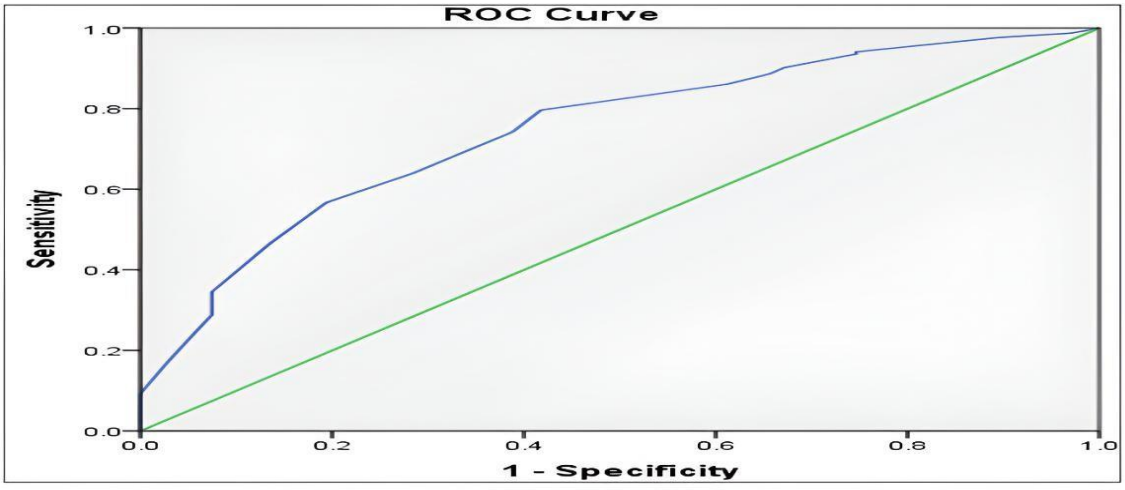

Figure-2: The Receiver Operating Characteristic (ROC) curve for the Cycle Threshold (Ct) values of RT-qPCR tests in predicting the survival outcomes

Optimal cut off value: 24.5
AUC: 0.746 (0.685–0.807), <p=0.00

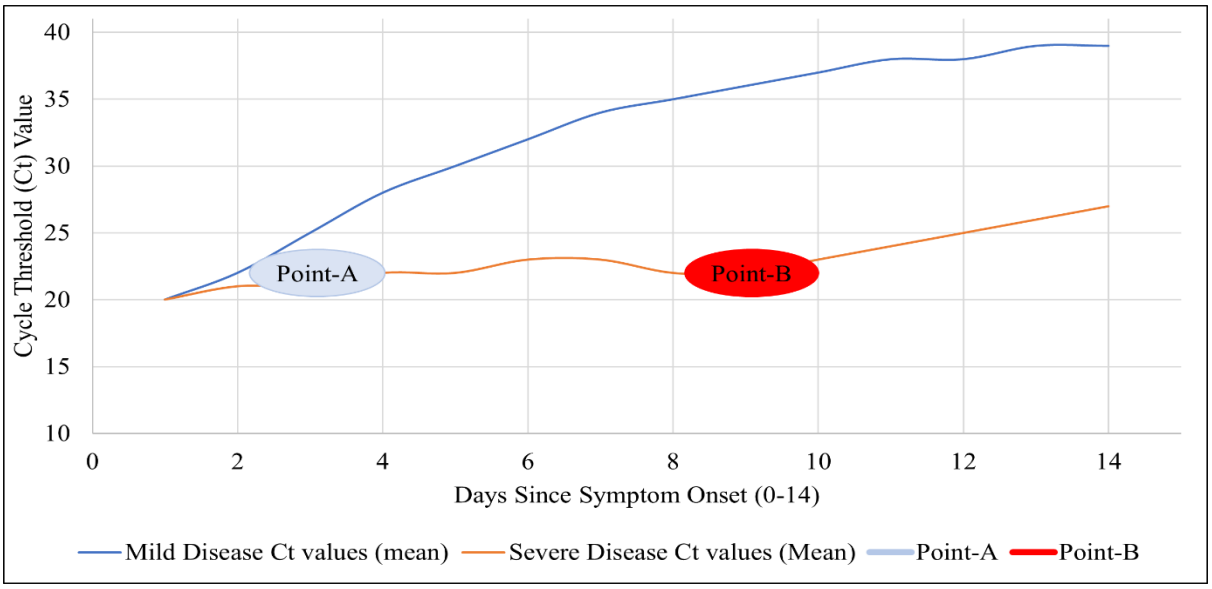

Figure 3. Conceptual model demonstrating confounding by symptom onset timing in Ct value interpretation.

Table 4: Model Summary for Multivariable Linear Regression (Ct as Dependent Variable)

Model R	R^2	Adjusted R^2	Std. Error of the Estimate
0.377	**0.142**	0.105	3.97

Despite these statistically significant univariate and multivariate findings, the comprehensive regression model explained a remarkably small portion of the variance in Ct values. The model R^2 was 0.142, and the adjusted R^2 was 0.105 (**Table 4**) which was indicating that the included clinical and laboratory variables accounted for only 14.2% of the observed variability in viral load, as estimated by Ct value of RT-qPCR.

This low explanatory power strongly suggests that one or more major, unmeasured variables were driving the majority of the variance in admission Ct values within this cohort.

Statistic	**Value**	**95% Confidence Interval**
Sensitivity	73.83%	64.45% to 81.85%
Specificity	88.79%	85.00% to 91.91%
Positive Likelihood Ratio	6.59	4.80 to 9.04
Negative Likelihood Ratio	0.29	0.21 to 0.41
Disease Prevalence	23.52%	19.69% to 27.69%
Positive Predictive Value	66.95%	59.61% to 73.54%
Negative Predictive Value	91.69%	88.90% to 93.83%
Accuracy	85.27%	81.68% to 88.40%

DISCUSSION

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df 1	df 2	Sig. F Change
1	.377[a]	.142	.105	3.9728129546	.142	3.795	19	435	.000

a. Predictors: (Constant), HR-CT, SPO2, CRP, CKD, WBC, loss of teste, COPD, breathlessness, HB, hypertension, diabetes, loss of smell, ferritin, IL-6, sore throat, LDH Creatinine, d-dimer, heart failure

Table-4: Summary of model

The model summary offers a full picture of the regression analysis and this will help to perceive its explanatory power and its importance in general. The model produced an R value of 0.377 that suggested that there was a strong moderate relationship between the independent variables that were set and the dependent variable. This amounts to approximately 14.2 percent variance of the outcome explained by the independent variables included in this model based on the value that the R Square takes, which is 0.142. Adjusted R square also declines to 0.105, thus considering the fit that could have been occasioned by several predictors. The standard error of estimate = 3.97, this is the value, which the observed values may deviate on the average of the predicted values.

The model significance testing gives a Sig. F Change of 0.000 and this means that overall model is statistically significant and the predictors as a group significantly influence the outcome variable. The change statistics indicate that the change of R Square of 0.142 is derived with F-value of 3.795 on 19 predictors with the degree of freedom of 435 that enhances the strength of the model. These results underline the importance of HR-CT, SPO2, IL-6, and creatinine as predictors of change in the dependent variable and the necessity to improve it and add more predictors (Table-4).

ANOVA[a]						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	1138.039	19	59.897	3.795	.000[b]
	Residual	6865.711	435	15.783		
	Total	8003.749	454			

a. Dependent Variable: RT-qPCR

b. Predictors: (Constant), HR-CT, SPO2, CRP, CKD, WBC, loss of teste, COPD, breathlessness, HB, hypertension, diabetes, loss of smell, ferritin, IL-6, sore throat, LDH Creatinine, d-dimer, heart failure

Table 5: ANOVA test

The ANOVA table just provides a closer examination of the analysis of variance carried on the regression model. The regression model has a total of squares of 1138.039 that represents the variation that is explained by the predictors. The high value of the regression mean square of 59.897 with 19 degrees of freedom shows the mean variation that it explains each of the predictors. The mean square of the residuals is 15.783 and the sum of squares of the residuals is 6865.711 divided by 435 degree of freedom of the residual. The Sum of Squares (S.O.S) of the model is 8003.749, which is an indication that the total summation of the regression and residual terms is equal to the total variation that is spotted in the dependent variable, RT_PCR. The calculated value of F-value is 3.795; this indicates the percentage of the amount of variance that has been explained as compared to the percentage of the variance that has not been explained. The test statistics indicate a p-value (Significance) of 0.000 that implies that the model is statistically significant at 95% level of confidence. It implies that the total of the predictors can cause the variations of RT_PCR results to an extremely large degree. The findings back the relevance of the study and highlights of the capacity of the proposed model to create meaningful contribution to the knowledge of the effects of the predictors on the dependent variable (Table-5).

In our analysis, we show how the application of standard statistical techniques, to a dataset with no information on the onset of any symptoms, can produce an interesting and false narrative on SARS-CoV-2 Ct values. We have found that there were statistically significant correlations between low admission Ct values and indicators of systemic inflammation (IL-6), organ dysfunction (creatinine), and hypoxemia. Moreover, ROC analysis proposed a moderate mortality discriminatory power (AUC=0.746). These results are reflections of the positive outcomes of many studies. Nonetheless, a critical analysis shows that they are essentially insufficient: an all-inclusive multivariate model accounting 19 clinical parameters could only explain 14.2 % of the variability in Ct values. This great lack of explanatory power is not simply a statistical accident, but a direct result of the failure to observe the most significant contextual variable.

A Ct value has no clinical meaning, except insofar as it is placed in the timeline of infection. Kinetic studies on viruses revealed that the maximum RNA load of the SARS-CoV-2 virus usually reaches the threshold of the early symptoms and then decreases. More importantly, the course varies based on the severity of the disease, the majority of the patients clear the virus effectively, and those with a transition to severe disease usually have delayed or even failed clearance causing high clearance of the virus (low Ct values) during the second week of the illness. Since we have no symptom onset data, and thus, two distinct populations with diametrically opposite prognoses are being combined: 1) patients presenting in their initial phase of the course (1-3 days) have a low Ct, and 2) patients presenting later (day 7-10 days) have a persistently low Ct, which is a sign of pathological viral persistence. The fact that our analysis and any analysis that does not account for this factor of confounding by time establishes a spurious association. It gives a false indication of prognostic meaning to a value which in most instances only indicates presentation at an early age as opposed to severity of disease (Figure. 3, Conceptual Model). Without the lost time data this confounding cannot be solved.

This interpretation can be supported by comparing our results with those that have been carried out methodologically rigorously and that did consider the onset of symptoms. Studies correcting or stratifying by duration of symptoms tend to discover the relationship between Ct value and outcomes attenuates, becomes insignificant, or turns in the opposite direction. Low Ct value might be correlated to higher results in case measured too early, but worse results in case measured later. This omission might be the reason behind the great heterogeneity and conflicting findings in the available literature. The findings of our study can be regarded as an in-vitro example of this phenomenon. We reproduced the usual methodological error and acquired the desired significant but ultimately meaningless sets of results.

The limitations of our study include its retrospective, single-centre design and the large number of patients that our study excluded because of non-availability of the data, which can have an impact on generalizability. The RT-PCR assay was specific to the RdRp and N genes; although the assays were of the same kind, the efficiency of primers/probes of the assays can affect the absolute Ct values. The most significant limitation, though, is the intentional and central one the lack of the data of the symptom onset. Other possible confounders (SARS-CoV-2 variant strain and vaccination) were not examined. These constraints do not undermine our argument but on the contrary demonstrate the kind of real world data constraints that render rigorous prognostic analysis of Ct values highly challenging.

CONCLUSION

Based on our findings, we must treat symptom onset as an essential baseline for any meaningful study of COVID-19 test results, not just an optional factor. To correct the current flawed research, we strongly urge that all future studies must account for the precise timing of symptoms when analyzing test data. Furthermore, scientific journals must make this a mandatory requirement for publication. Until this vital standard is adopted, the existing research cannot be reliably used to guide real-world medical decisions.

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