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Original article

Rethinking of the clinical utility of the cycle threshold value of the RT-qPCR of COVID-19 in decision-making and prediction of the patients' outcomes

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ABSTRACT

Background: The cycle threshold (Ct) values derived from real-time quantitative PCR (RT-qPCR) testing for COVID-19; a widely utilized diagnostic tool holds the potential as a prognostic marker. However, its clinical utility in guiding decision-making and improving patients' outcomes is questioned. **Aim:** This study critically examined the Ct values' role in assessing COVID-19 severity, and outcomes, addressing gaps and inconsistencies in the current interpretations and applications of this test, to contribute in decreasing the morbidity and mortality of the disease. **Materials and methods:** This cross-sectional, prospective, single-center study was done on 158 COVID-19 patients divided into three groups based on Ct values; moderate to high viral load (Ct < 30), low viral load (Ct =30 -40) and undetectable (Ct >40 or not detected). Patients' data including demographics, symptoms, risk factors, vital signs, disease severity, and laboratory investigations were all collected. **Results:** Non-significant association was detected in the three groups between Ct values and factors such as age, sex, clinical symptoms, smoking habits, or disease severity, notably headache and renal diseases were more frequent in the low viral load group while total white blood cells and lymphocytes counts were significantly lower in positive groups than in the undetectable group, also Ct values weren't significantly correlated with patient outcomes. **Conclusion:** cycle threshold values of the RT-PCR testing, which are negatively associated with viral load, are not a convincing COVID-19 indicator for severity and outcome, despite some correlations being found.

Introduction

The Cycle threshold (Ct) parameter is the cycle number required in the Reverse transcriptase quantitative real-time PCR (RT-qPCR) assay to amplify viral nucleic acid so it can reach a detectable level to be recorded for each sample [1]. The sample's target concentration of viral nucleic acid is negatively correlated with the Ct value [2]. Ct value-based viral load estimates have been utilized to predict illness development, determine transmissibility, and distinguish between active viral reproduction and protracted virus shedding

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[3]. Reduced Ct levels are correlated with increased lactate dehydrogenase (LDH) levels and lower lymphocyte levels, which are linked to higher severity and poor prognosis in COVID-19 cases [4,5].

Some researchers consider Ct values reported by the presently accessible RT-qPCR tests do not accurately match RNA levels and are inconsistent across platforms; hence, Ct values should not be used in clinical decision-making [6].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections have 2 stages, firstly the entrance of the virus in the tissues and cells which cause the main symptoms, secondly the "cytokine storm" which is a systemic inflammatory stage associated with release of numerous inflammatory markers which is responsible for disease severity, complications and outcome [7].

Most individuals with COVID-19 are presented only with mild symptoms (85%), including fever, headache, fatigue, myalgia, rhinorrhea, anosmia, diarrhea, cough, and sputum. Severe cases (15%) include dyspnea, tachypnea (respiratory frequency > 30 breaths/minute), pneumonia with hypoxemia, cyanosis, hypotension, and lymphopenia, and lung infiltrates > 50% of patients. Sever disease must be treated in a hospital bed. The mortality rate for COVID-19 is around 2.9% [8].

Most adult patients with COVID-19 have a good prognosis, but the patients aged ≥ 60 years and those with chronic underlying diseases such as chronic respiratory disease, cardiovascular disease, diabetes, obesity, and hypertensive heart disease, are at a greater risk for developing a severe or critical illness from COVID-19. The severity of the diseases is directly related to poor clinical outcomes. In addition, the time interval between symptom onset and death is shorter among elderly patients (≥ 65 years) [9].

The WHO classified the clinical severity of COVID-19 patients as asymptomatic, mild, moderate, severe, and critical ill [10].

Although COVID-19 is now in the post-pandemic phase, it is still associated with thousands of hospitalizations and hundreds of deaths each week in the United States and can lead to long COVID [11]. Even in the post –pandemic era, the interpretation of Ct values continues to hold relevance in specific clinical scenarios, e.g. immunocompromised patients, outbreaks in healthcare facilities, and diagnosis of infection during respiratory viral seasons [12].

This study aimed to find the relation of the Ct value of RT-qPCR for SARS-CoV-2 with the different clinical data, laboratory data, and outcomes of COVID-19 cases to decrease morbidity and mortality.

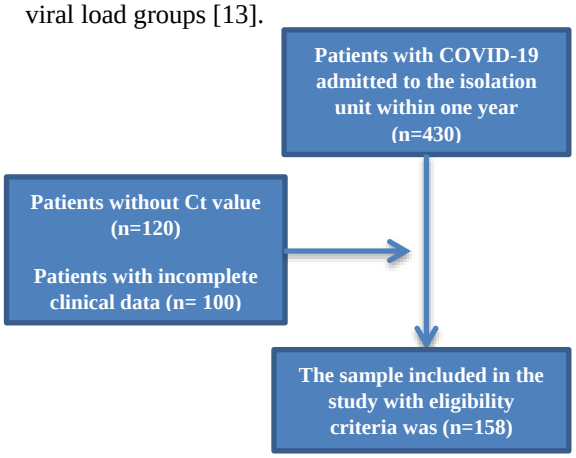
Subjects, material and methods

Study location and design

This prospective, cross-sectional, and single-center study was done in the isolation units for COVID-19 patients and in the Scientific and Medical Research Centre, Faculty of Medicine, Zagazig University, from February 2021 to January 2022. The study has been approved by the Institutional Review Board (IRB) and Ethical Committee of the Faculty of Medicine, Zagazig University (IRB#: 6421-23-9-2020). It was conducted according to the Helsinki revised declaration. Consents were obtained from patients or their accompanying people upon sample collection.

The subjects

The sample of the study was collected as consecutive sampling using all accessible patients in the study period who fulfilled the inclusion criteria. The Patients included in this study were confirmed COVID-19 patients, with available and complete clinical and laboratory data, and the RT-qPCR test for detection of SARS-CoV-2 was implemented for them in our institute at the Scientific and Medical Research Center, Faculty of Medicine, Zagazig University for the detection of Ct values. Any patient who was diagnosed only clinically and SARS-CoV-2 RT-qPCR was not implemented for him or with incomplete clinical or lab data or who refused to participate in the study was excluded. A total of 158 cases were included in this study. The included patients were classified according to the Ct value of SARS-CoV-2 RT-PCR test result into; undetectable, low viral load, and moderate to high viral load groups [13].



Clinical and laboratory data collection

The patients' data were obtained from their medical records, which included: (name, age, and sex), symptoms such as (fever, cough, fatigue, diarrhea and dyspnea), the clinical risk factors data;(hypertension, diabetes mellitus, renal disease, hepatic disease, and cardiac disease), the vital signs were recorded for each patient, including (blood pressure [BP], heart rate [HR], respiratory rate [RR], Oxygen saturation [SaO2], Temperature and conscious level). The type of ventilation used; either (continuous positive airway pressure [CPAP], reservoir, or ventilator), and lastly; the severity of the disease in COVID-19 which was determined according to the WHO interim guidance for Clinical management of COVID-19, and as we were dealing with hospitalized patients; the included patients were either moderate, severe, or critically ill COVID-19 cases [14].

For all the included patients, the following investigations were performed (differential WBC count, CRP, LDH, D-dimer, serum Ferritin, Procalcitonin, blood culture, and sputum culture). The clinical outcome was recorded, either survival or death. Patients were treated according to the Egyptian management guidelines for COVID-19 (Ministry of Health and Population 2020) [15].

SARS-CoV-2 RT qPCR test

Qualified staff members at the hospital's isolation units collected samples, Personnel were trained in uniform collection techniques to ensure consistency across samples, which formed of a nasopharyngeal (NP) and an oropharyngeal swab (OP) [16]. Both were add-mixed in a 3-ml tube of a viral transport medium (VTM-tube, Ismailia free zone, Egypt. Ref: 1/V T01.001.0001, lot no. BSVT45620). Samples were transported to the laboratory within 30 minutes of collection at a temperature less than 5°C and processed immediately or stored at -20 °C to preserve integrity of samples. Non-duplication of samples was considered.

The samples were tested using the COVID-19 Genesig RT-PCR assay for SARS-CoV-2 (Primerdesign Ltd, COVID-19 Genesig® Ref: Z-COVID-19-CE, Lot: JN-02780-0145m, School Lane,Chandler Ford, UK) after employing QIAamp® Viral RNA Mini Kit for RNA extraction (Qiagen Strasse, Helden, Germany. Ref: 52906, Lot: 166027658). The experiment comprised an RNA internal extraction control, a negative amplification control using nuclease-free water, and a positive control template. The reaction mix was 20ul (10ul of oasig™ One Step 2X RT-qPCR Master Mix, 2ul of COVID-19 Primer & Probe, and 8ul of the sample extract); its target gene is the RNA-dependent RNA polymerase (RdRP); the manufacturer reports a detection limit of 0.58 copies/µl. The thermal profile was ten minutes at 55°C, two minutes at 95°C, and then 40 cycles of 10 seconds at 95°C and one minute at 60°C. The same kits were used throughout the study to ensure consistency of results.

Positive samples were defined as those with a Ct value ≤40 and an exponential development curve, they were classified as moderate-high viral load with a Ct value (<30) and low viral load with a Ct value (30-40), while the Ct values >40 or no Ct values detected were considered as undetectable [13]. The undetectable samples included in this study were clinically diagnosed as COVID-19 cases and had previous positive RT-qPCR for SARS-CoV-2, but the test became negative or undetectable at the time of sample collection for our study. The qRT-PCR Ct values were gathered. It exhibits an exponential and inverse relationship with the virus's copy number: as the viral RNA concentration increases, the Ct value decreases in an exponential manner. The apparatus used for the RT-PCR assay was Stratagene Mx3005P (Agilent, Santa Clara, United States). All test procedures were performed under a BSL2 cabinet.

Statistical analysis

SPSS software Version 20.0 (Statistical Package for Social Science) was used for data analysis. Categorical variables were represented as numbers and percentages. The Kruskal-Wallis H test and one-way ANOVA were used to determine differences in continuous variables, while the Chi-square was used to compute differences in categorical variables. A p-value < 0.05 is considered statistically significant.

Results

During the one-year study period, 430 COVID-19 cases were admitted to the COVID-19 isolation unit at Zagazig University hospitals: only 158 cases were included in the current study which fulfilled the inclusion criteria; 81 cases had Ct value <30, so included in the moderate to high viral load group, 45 cases had Ct value = 30-40, so included in the low viral load group and 32 cases had Ct value ≥40 or no Ct value detected, so included in the undetectable group.

As **table 1** shows, no statistical significance was detected between the three studied groups regarding age, gender, the symptoms including (dyspnea, fatigue, fever and diarrhea), smoking habit, the risk factors (including hypertension, diabetes mellitus, hepatic disease, and cardiac disease), the type of ventilation used with the patients and the clinical severity of the disease. On the other hand, headache and renal disease were significantly more presented in the low viral load group than in the other 2 groups (p-value = 0.02, 0.01) respectively.

Concerning the vital signs presented in **table 2**; a nonsignificant difference was detected between the 3 studied groups.

As regards the lab investigations presented in table 3; a significant decrease in total WBCs and lymphocytic cell counts in the 2 positive groups in comparison with the undetectable group (p-value = 0.002, 0.05) respectively. Non-significant difference was detected in the other lab parameters in the 3 studied groups.

As presented in **figure 1**; no statistically significant difference was detected between the Ct value and the patients' outcomes in the studied groups; the total number of survived patients were 84; distributed as18 (56.3%), 23 (51.1%) and 43 (53.1%) in the undetectable Ct value group, the low viral load group and the moderate to high viral load group respectively.

Table 1. The Relation between the Ct value and (the demographic data and factors related to the disease).

Ct value / Patient factor	**Undetectable (>40) (N=32)**	**Low viral load (30-40) (N=45)**	**Moderate-high viral load (<30) (N=81)**	***P* value**
Age (years)*				
Mean ± SD	63.7±14.29	61.8±15.37	58.73±15.45	0.31
Range	(23-90)	(20-85)	(17-95)	
Gender **				
Male	14(43.8)	26(57.8)	41(50.6)	0.47
Female	18(56.2)	19(42.2)	40(49.4)	
Symptoms**				
Dyspnea	32(100.0)	45(100.0)	81(100.0)	----
Fatigue	0(0.0)	1(2.2)	5(6.2)	0.24
Fever	16(50.0)	13(28.9)	31(38.3)	0.17
Headache	0(0.0)	3(6.7)	0(0.0)	0.02
Diahrrea	1(3.1)	0(0.0)	1(3.1)	0.48
Smoking habit**				
Yes	8(25.0)	14(31.1)	24(29.7)	0.83
No	24(75.0)	31(68.9)	57(70.3)	
Clinical risk factors **				
Hypertension	10(31.3)	14(31.1)	33 (40.7)	0.45
Diabetes mellitus	16(50.0)	16(35.6)	26(32.1)	0.2
Renal disease	2(6.3)	9(20.0)	4(4.9)	0.01*
Hepatic disease	1(3.1)	5(11.1)	5(6.2)	0.36
Cardiac disease	2(6.3)	2(4.4)	1(1.2)	0.33
Type of ventilation **				
CPAP	11(34.4)	14(31.1)	26(32.1)	0.14
Reservoir	14(43.8)	28(62.2)	49(60.5)	
Ventilator	7(21.9)	3(6.7)	6(7.4)	
Clinical Severity**				
Moderate	18(56.2)	34(75.6)	49(60.5)	0.14
Severe and critically ill	14(43.8)	11(24.4)	32(39.5)	

*one way ANOVA, ** Chi-square test

Table 2. The Relation between Ct value of and vital signs of patients.

Ct value / Vital signs	Undetectable (>40) (N=32)	Low viral load (30-40) (N=45)	Moderate-high viral load (<30) (N=81)	*P* value
Oxygen Saturation (%)*				
Mean ± SD	75.8±9.5	78.2±7.86	77.34±9.2	0.52
Range	(50-89)	(60-90)	(45-90)	
Systolic blood pressure (mmHg)*				
Mean ± SD	124.37± 18.08	126.8±16.4	128.39± 19.6	0.57
Range	(85-170)	(100- 170)	(75-190)	
Diastolic blood pressure (mmHg)*				
Mean ± SD	75.0±15.86	75.22± 11.72	78.39±14.82	0.35
Range	(30-100)	(50-100)	(40-120)	
Respiratory rate*				
Mean ± SD	28.18±1.68	26.86± 2.65	28.2±11.7	0.61
Range	(23-35)	(20-35)	(20-130)	
Heart rate*				
Mean ± SD	93.21±18.45	91.5±15.82	91.45±18.8	0.8
Range	(51-145)	(62-140)	(58-190)	
Temperature*				
Mean ± SD	37.64± 0.58	37.45± 0.53	37.5±0.6	0.36
Range	(36-39.4)	(36.3-39.5)	(36.4-39)	
Conscious level **				
Conscious	28(87.5)	42(93.3)	75(92.6)	0.6
Unconscious	4(12.5)	3(6.7)	6(7.4)	

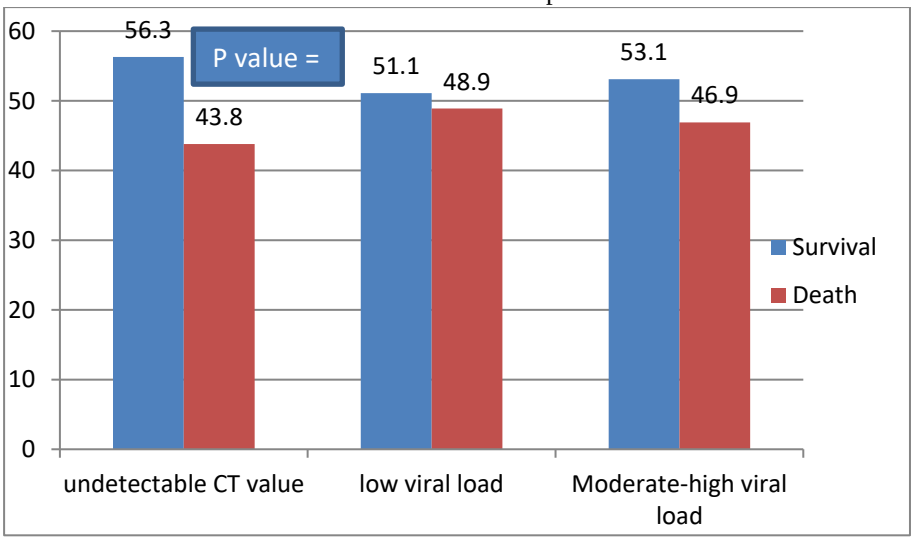
*One-way ANOVA, ** Chi-square test

Table 3. The relation between the Ct value and the other laboratory findings.

Ct value / Lab findings	Undetectable (>40) (N=32)	Low viral load (30-40) (N=45)	Moderate-high viral load (<30) (N=81)	*P* value
WBCs * Median Range	15.45 (7-65)	10.8 (3.6-28.6)	10.6 (1.1-35.0)	0.002*
Neutrophil* Median Range	13.5 (5.4-60)	10 (1.6- 25.6)	8.9 (0.7-150.0)	0.34
Lymphocyte* Median Range	0.95 (0.2-2.7)	0.6 (0.0-2.4)	0.6 (0.0-3.4)	0.05
CRP* Median Range	124 (28.0-345.0)	132 (1.0-441)	124 (2.3-404)	0.5
LDH* Median Range	506 (200-980)	427 (0.2-718)	487.5 (92-1650)	0.4
Procalcitonin* Median Range	0.72 (0.02-7.3)	0.17 (0.02-20)	0.18 (0-55.0)	0.76
D dimer* Median Range	1.2 (0.3-10.3)	1.2 (0.20-8.2)	1.2 (0.2-16.0)	0.67
Ferritin* Median Range	1304 (116-1999)	1254 (67.0-8050)	1222 (144-5721)	0.76
Blood Culture** No organism *Klebsiella sp.* *Staph hominis* *E-coli* Fungal	22(84.6) 3(11.5) 0(0.0) 1(3.8) 0(0.0)	42(97.7) 0(0.0) 1(2.3) 0(0.0) 0(0.0)	65(91.5) 3(4.2) 1(1.4) 1(1.4) 1(1.4)	0.37
Sputum Culture** No organism *Klebsiella sp.* *Enterococci sp.* *E-coli sp.*	19(76.0) 4(16.0) 1(4.0) 1(4.0)	24(75.0) 7(21.9) 0(0.0) 1(3.1)	44(68.8) 15(23.4) 0(0.0) 5(7.8)	0.48

*Kruskal Wallis test ** chi-square test

Figure 1. The relation between Ct value and the outcome of the patients.



Discussion

The Ct value in the RT-qPCR test is illustrated as the "number of cycles needed for an amplicon to be identifiable above baseline". An increased quantity of target viral sequences in the tested sample correlates with decreased Ct results [17].

Evidence from the SARS-CoV epidemic of 2002 indicated that higher viral load was associated with the increased need for intensive care and an overall worse prognosis [18]. The worth of the PCR Ct values in treating COVID-19 cases and its consequences are still up for debate [19].

To the best of our knowledge, it is the first study implemented in our country to gain a better understanding of the relationship between Ct levels and COVID-19 disease severity, prognosis, and outcome.

In the present study, the mean age of cases in undetectable, low viral load, and moderate to high viral load groups were 63.7±14.29, 61.8±15.37, and 58.73±15.45, respectively, without significance between them (p= 0.31) (table 1). This was agreed with **Dergaa et al.** [20], who found no correlation between age and viral load, but in contrast to **Zhao et al.** [21] who noticed that cases who were 80 years or older at diagnosis had a significantly greater viral load than other cases,

Regarding the symptoms of the studied subjects, they mostly presented with dyspnea and fever with no significant difference between the three studied groups. Other symptoms including headache, fatigue, and diarrhea which were presented commonly at the beginning of the disease were detected in only a few cases of the studied subjects; headache; was presented in 3 cases only in the low viral load group, fatigue; was presented in 1 case of the low viral load group and 5 cases in the moderate to high viral load group, and diarrhea; 1 case in the undetectable group and 1 case in the moderate to high viral load group **(Table 1).** This pattern of results is consistent with **Jemmieh et al.** [22], who stated that when categorized, Ct value (< 25 vs ≥ 25) had no association with the odds of ICU admission (OR 0.85, 95% CI 0.56 to 1.29) or odds of respiratory (OR 2.95, 95% CI 1.57 to 5.56) and gastrointestinal symptoms (OR 1.99, 95% CI 1.18 to 3.35). Whereas other researchers have found significant differences in symptoms among patients with low, medium, and high Ct values [23].

There was no substantial variation between the three Ct value groups for risk factors such as smoking, hypertension, diabetes, hepatic, and cardiac patients. Still, in renal patients, the difference was statistically significant **(Table 1),** and **Ashrafi et al.** [24] agreed, revealing that Ct values were elevated in cases with chronic kidney disease than in non-renal patients (p-value 0.01).

On the other side, as regards the type of ventilation, either reservoir, CPAP, or Ventilator for the three studied groups, no statistical significance (p-value 0.14) was detected (table 1), and this agrees with **Barry et al.** [25] and **He et al.** [26] who revealed that there was no statistical significance between Ct values and need for oxygenation and disease severity. Whereas past researchers, **Zhao et al.** [21], revealed that non-survivor patients with higher viral load needed more oxygenation than survivors with lower viral load.

In our study, we found that there was no statistical significance between the Ct value in the three studied groups and COVID-19 severity (p-value 0.14) **(Table 1).** This finding provides supporting evidence that Ct values do not play a predictive role in clinical settings, and this was in agreement with **He et al.** [26] and **Lee et al.** [27], who stated that there was no clear variation in viral loads across illness severity. Others like **Liu et al.** [28], **To et al.** [29], and **Schwierzeck et al.** [30] revealed that Ct values were negatively correlated to disease severity.

Interestingly, the vital signs and the clinical data of the patients in the three groups including temperature (p= 0.36), HR (p= 0.8), oxygen saturation (p= 0.52), systolic blood pressure (p= 0.57), diastolic blood pressure (p= 0.35), respiratory rate (p= 0.61), and conscious level (p= 0.6) **(Table 2)** showed no statistically significant difference. This did not agree with **Dergaa et al.** [28], who found that greater viral load was related to greater heart rate, increased temperature, and reduced oxygen saturation. These findings were similar to **Camargo et al.** [27], who found no relation between oxygen saturation, oxygen requirement and Ct values at admission.

In this study, a significant decrease in the total white blood cell counts and lymphocytic count in the low viral load and moderate to high viral load groups of Ct values in comparison to the undetectable Ct value group. (p-value 0.002 and 0.05, respectively) **(Table 3).** These findings were in partial agreement with **Yuan et al.** [31], who

inversely correlated Ct with neutrophil and lymphocyte counts but different from **Liu et al.** [28], who negatively correlated Ct values with neutrophil and lymphocyte counts.

Furthermore, the concentrations of the inflammatory factors (such as LDH, CRP, Procalcitonin Ferritin, and D dimer), showed no statistical significance between the three Ct values groups (*p*-value 0.5, 0.4, 0.76, 0.76 and 0.67 respectively) **(Table 3)** and this was similar to **Yuan et al.** [31] but not in agreement with **Liu et al.** [28] and **Azzi et al.** [32] who found negative correlation between Ct values and inflammatory markers which assumed that The incidence of severe COVID-19 was closely associated with inflammatory response and coagulation disruption.

In our study, we found no statistically remarkable association between the positive results of the blood and sputum cultures or the type of the isolated microorganism and the three groups of Ct values (*p*= 0.37 and 0.48, respectively) **(Table 3),** but that was different from La Scola et al. [33], who reported a correlation between the rate of positive cultures and Ct value.

In our study, we found no statistical significance with patients' outcomes either survival or death (*p*-value 0.9) **(Figure 1)** in different Ct values groups, and this was in line with, **Abdulrahman et al.** [34] who found that disease outcome has no significant relation with Ct values and also, **Camargo et al.** [19] detected no significant relationship between Ct levels and severity or mortality outcomes. However, these findings did not agree with **Huang et al.** [35], who found that the mean Ct levels decreased in death cases compared to those not discharged.

Finally, our results could be explained by the idea that the Ct values could be influenced by different factors like; the individual host response, the timing of testing or the quality of specimens, which in turn affect the RT-qPCR results.

Conclusion

While Ct values correlate with viral presence, their predictive power for disease severity, laboratory data, clinical outcomes, or mortality is limited. These findings highlight that relying solely on Ct values for decision-making can be misleading, particularly in heterogeneous patient groups.

Limitations of the study

In our study we did not include the COVID-19 patients who were asymptomatic or with mild symptoms, also the timing of hospitalization in relation to disease period and the timing of sample collection in relation to the onset of symptoms were not included in our data which may affect the Ct value of the patients, Multi-center studies on larger number of patients were needed to confirm our results. Certain preanalytical factors, such as variability in sample quality or unavoidable delays during transport—may still affect Ct values. These factors are inherent limitations in Ct-based studies.

Conflicts of interest

Authors declare that they have no conflicts of interest.

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