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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Is There a Correlation Between the Cycle Threshold of SARS-CoV-2 RT-PCR and the Clinical Course of COVID-19?

SARS-CoV-2 RT-PCR'nin Döngü Eşiği ile COVID-19'un Klinik Seyri Arasında Bir Korelasyon Var mıdır?

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Abstract

Objectives: Many parameters are studied in coronavirus disease-2019 (COVID-19) to predict the progress of the disease. One of these parameters is the clinical significance of the reverse transcriptase-polymerase chain reaction (RT-PCR) cycle threshold (CT) value used in diagnostic tests. In this study, we evaluated the relationship between RT-PCR CT values and the clinical course of COVID-19.

Materials and Methods: Symptomatic patients over the age of 18 years, who had positive severe acute respiratory syndrome-coronavirus-2 RT-PCR test results between June 1, 2020 and December 1, 2020, were screened retrospectively. Patients' CT values and other data were collected from the hospital's information management system.

Results: The study included the data of 880 patients. The median age was 63 years, and 47% (415) were female. The severity of COVID-19 was mild in 69.7% (614), moderate in 20.4% (180), and severe/critical in 9.7% (86). There was no significant difference between median CT (mCT) levels of disease severity (mCT=22 in mild group; mCT=23 in moderate group; mCT=22 in severe/critical group, p=0.882). The results showed no correlation between these CT values and COVID-19's severity, prognosis, or laboratory values.

Conclusion: Although there are some reports that propose a relationship between CT values and viral load, we believe that these test results cannot be considered quantitative and cannot be generalized because of the many factors known to affect CT values.

Key Words: Viral Load, Cycle Threshold, COVID-19

Öz

Amaç: Hastalığın progresyonunu tahmin etmek için koronavirüs hastalığı-2019'da (COVID-19) birçok parametre incelenmektedir. Bu parametrelerden biri, tanısal testlerde kullanılan ters transkriptaz-polimeraz zincir reaksiyonu (RT-PCR) döngü eşiği (CT) değerinin klinik önemidir. Bu çalışmada RT-PCR CT değerleri ile COVID-19'un klinik seyri arasındaki ilişkiyi değerlendirdik.

Gereç ve Yöntem: 1 Haziran 2020 ile 1 Aralık 2020 tarihleri arasında şiddetli akut solunum sendromu-koronavirüs-2 RT-PCR testi pozitif çıkan 18 yaş üstü ve semptomatik hastalar geriye dönük olarak tarandı. Hastaların CT değerleri ve diğer verileri hastanenin bilgi yönetim sisteminden toplandı.

Bulgular: Çalışmaya 880 hastanın verileri dahil edildi. Hastaların yaş ortancası 63 idi ve %47'si (415) kadındı. COVID-19 hastalığının şiddeti hastaların %69,7'sinde (614) hafif, %20,4'ünde (180) orta ve %9,7'sinde (86) şiddetli/kritik idi. Medyan CT (mCT) değerleri ile hastalık şiddeti arasında anlamlı bir fark yoktu (hafif hastalık grubunda mCT=22; orta hastalık grubunda mCT=23; şiddetli/kritik hastalık grubunda mCT=22, p=0,882). Çalışmamızın sonuçları, CT değerleri ile COVID-19'un şiddeti, prognozu veya laboratuvar değerleri arasında bir ilişki olmadığını gösterdi.

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Sonuç: Pratikte CT değerleri ile viral yük arasında göreceli bir ilişki olmasına rağmen, CT değerlerini etkilediği bilinen birçok analitik ve klinik faktör nedeniyle bu test sonuçlarının nicel olarak kabul edilemeyeceğine ve genellenemeyeceğine inanmaktayız.

Anahtar Kelimeler: Viral Yük, Siklus Eşiği, COVID-19

Introduction

The clinical course of coronavirus disease-2019 (COVID-19) can vary from asymptomatic disease to severe respiratory failure with serious sequelae and even fatal outcomes. Being able to predict patients' prognosis at diagnosis can greatly assist patient-management decisions. The parameters that clinicians can use to predict the course of COVID-19 do not occur in the same way in all patients, and even poor prognosis markers do not show a bad prognosis for every patient (1).

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) reverse transcriptase polymerase chain reaction (RT-PCR) test is used for diagnosis, screening, and surveillance of COVID-19, and the results are usually reported to the referring physician as either positive or negative. However, the test's cycle threshold (CT) value might provide a measure of the viral load in the sample. In RT-PCR, the CT values represent the number of amplification cycles required for the target gene to exceed a threshold level. Therefore, CT values are inversely proportional to the viral load and can provide an indirect method of measuring the number of copies of viral RNA in a sample. Low CT values may be associated with high viral loads. Studies in the literature indicate that the SARS-CoV-2 viral load may be used as a parameter that can determine the disease's severity and prognosis (2-4).

In this study, we examined the relationship between the CT values of patients diagnosed with COVID-19 using RT-PCR and those patients' demographic characteristics, clinical courses, and poor prognostic markers in laboratory values.

Materials and Methods

Patient Characteristics

This retrospective cross-sectional study screened patients over age 18 years who had RT-PCR test results that were positive for COVID-19 at Başkent University Hospital between June 1, 2020 and December 1, 2020. Patients whose samples tested positive before surgery or interventional procedures were considered asymptomatic and excluded from the study.

The patients' data; demographic characteristics (age, gender, comorbidities, use of immunosuppressive), symptoms on admission (fever, shortness of breath, cough, sore throat, headache, myalgia, loss of taste and smell, diarrhea), duration of symptoms, laboratory values on admission [lymphocytes, C-reactive protein (CRP), ferritin, D-dimer] were retrieved by an infectious diseases specialist from the hospital's information management system. Assuming that the CT value represents viral load, we assessed the severity of the disease based on the symptoms at presentation. The severity of COVID-19 as experienced by patients was graded as mild, moderate, and severe/critical, according to the World Health Organization classification (5). We looked at the patients' prognosis in the 30-day period following a positive PCR test. The patients' hospitalization, intensive care unit support, need for mechanical ventilation, and mortality were retrieved from their medical records. Some patients COVID-19 prognosis who were referred to the pandemic hospital were retrieved from the database of Turkey's national public health management system.

Poor prognostic markers as defined by the COVID-19 guideline of the Turkish Ministry of Health included blood lymphocyte count <800/µL, CRP>50 mg/L, ferritin>500 ng/mL, and D-dimer>1000 ng/mL (6). Only the laboratory values of the patients who were tested concurrently with the SARS-CoV-2 PCR in the application were evaluated.

The status of receiving antiviral treatment of patients was checked and it was confirmed that they did not have. At the time of the study, COVID-19 vaccination had not yet begun in our country.

This study was approved by the Başkent University Institutional Review Board (project no: KA20/145, date: 12.01.2021). In organizing the study, the Strengthening the Reporting of Observational Studies in Epidemiology rules were followed.

RT-PCR Test

Our hospital's PCR laboratory is authorized as a COVID-19 diagnostic laboratory by the Turkish Ministry of Health. On April 29, 2020, our laboratory began to diagnose COVID-19 using the Bio-Speedy® Direct RT-qPCR SARS-CoV-2 test (Bioeksen, Turkey), which was approved and offered for use by the Turkish Ministry of Health. The sensitivity of the Bio-speedy® Direct RT-qPCR SARS-CoV-2 kit was determined as 97.8% and the specificity as 100% (7).

This test achieves rapid results using one-step reverse transcription and real-time PCR that targets fragments of the *ORF1ab* and *N* genes. To screen for COVID-19, combined oropharyngeal and nasopharyngeal samples were taken using swabs in COVID-19 outpatient clinics and from suspected hospitalized cases and transmitted to the laboratory in viral transport medium (VTM). For each patient, a 20-µL mixture was prepared by taking 5 µL of sample from the VTM and mixing

it with 10 µl of 2X Prime Script Mix and 5 µl of Di Oligo Mix. This sample was put into a Rotor-Gene Q 5plex High-Resolution Melt analyzer (Qiagen), and the appropriate program was selected, yielding results in 90 minutes. The determination of clinical sample test results was evaluated in conjunction with positive and negative control growth curves. For Rotor-Gene Q 5plex, the threshold level recommended for calculating CT values is 0.02 relative fluorescence units. If the CT value is < 38, it is interpreted as positive, and if it is ≥38, it is interpreted as negative.

Statistical Analysis

During the statistical evaluation, the conformity of the numerical data to the normal distribution was evaluated using graphical methods and the Kolmogorov-Smirnov test. The data that had parametric properties were expressed as mean ± standard deviation (mean ± SD), and a Student's t-test was used to compare two independent groups. The data that did not have parametric properties were shown as median and interquartile range (IQR), and the Mann-Whitney U test was used to compare two independent groups. When assumptions were met, the Kruskal-Wallis test or a one-way ANOVA were used to compare more than two independent groups. The nominal data were expressed as numbers (n) and percent (%), and group comparisons were made using chi-square or Fisher's exact tests, as appropriate. Statistical analyses were performed using IBM® SPSS© version 25 software (Armonk, NY: IBM Corp.). To determine statistical significance, the type-1 error level used was 5%.

This study was approved by the Başkent University Institutional Review Board (project no: KA20/145).

Results

One thousand four hundred and eighty-two of 13,869 respiratory samples were positive at our institution between June 1 and December 1, 2020. Four hundred thirty of the 1,482 patients were under the age of 18, and 15 had a positive result at surgical screening. Duplicate data were found in 62 patients. In addition, 11 samples were collected from the lower respiratory tract. Furthermore, the data of 84 patients could not obtained (Figure 1). Therefore, the study included 880 patients, 47.2% (415) of whom were women.

While the median age of all patients was 63 years (minimum 18, maximum 100), the median age in the severe/critical disease group was 72.5 years. The disease's severity was mild in 69.7% (614), moderate in 20.4% (180), and severe/critical in 9.7% (86). Hypertension was the most common comorbidity, at 16.7% (147); followed by diabetes mellitus, at 8.3% (73); and cardiovascular disease, at 7.3% (64). All the comorbidities were more likely in the severe/critical disease group than in the other groups (p<0.001). Table 1 shows the patients' demographic characteristics.

In all disease severity groups, at hospital admission, the median symptom day was 3 days. The most common presenting symptom was malaise (51.2%, 451), followed by fever (48%, 422), and cough (40.3%, 355). Of the 98 patients who presented with dyspnea, 48 (55.8%) were in the severe/critical illness group.

There was no significant difference between median CT (mCT) levels of disease severity [mCT=22 (IQR 19-25) in mild group; mCT=23 (IQR 18-25) in moderate group; mCT=22 (IQR 18-25) in severe/critical group, p=0.882]. When poor

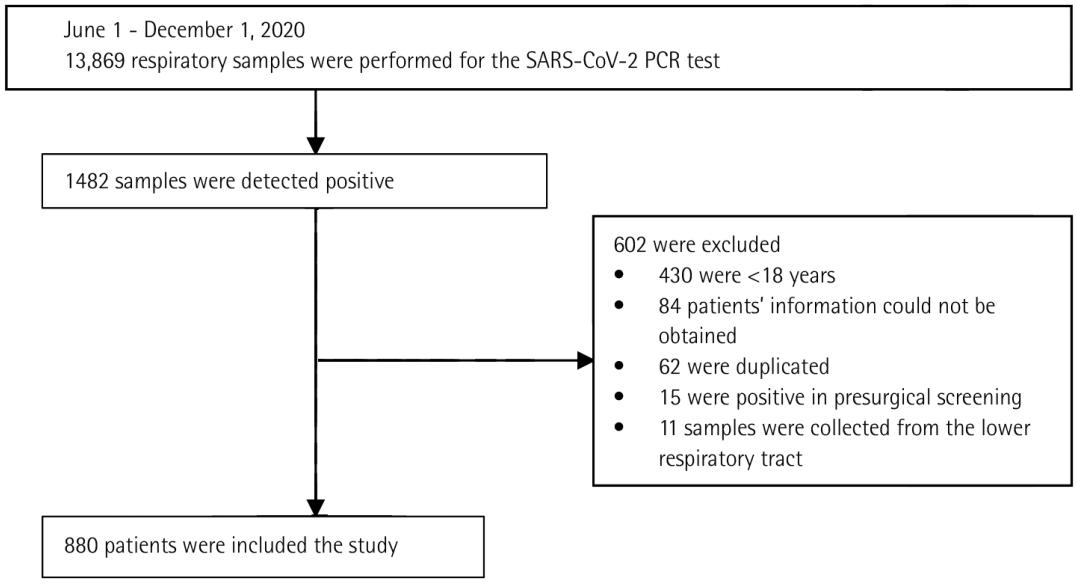

Figure 1: Study flowchart

SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, PCR: Polymerase chain reaction

prognostic markers were examined in the 534 patients who had laboratory data assessed at admission, a significant correlation with disease severity was found (p<0.001). Thirty-five (41.2 percent) of the 77 patients with lymphopenia were classified as severe/critical. The severe/critical disease group had greater CRP, D-dimer, and ferritin levels than the other groups. Table 2 is illustrated the laboratory values of patients based on the disease severity.

Thirty (3.4%) patients needed mechanical ventilation, and 50 (5.7%) patients died. Twenty patients had died without mechanical ventilation, due to sudden death.

To better demonstrate the RT-PCR CT values, we divided them into 4 groups according to their quartiles: Q1 (CT≤18), Q2 (CT=19-22), Q3 (CT=23-25), and Q4 (CT≥26). We found no relationship between these CT groups and either age,

Table 1: Demographic characteristics

Variables	All n=880	Mild n=614	Moderate n=180	Severe/critical n=86	p-value
Age median (IQR)	63 (50-74)	39 (29-49)	53.5(43-68)	72.5 (61-79,2)	<0.001*
Gender Female n (%)	415 (47.2)	303 (49.3)	75 (41.7)	37 (43)	0.139
Duration of symptoms median (IQR)	3 (2-4)	3 (2-4)	3 (2-4)	3 (1-6.7)	0.651
Comorbidities Yes n (%)	241 (27.1)	91 (14.8)	86 (47.8)	64 (74.4)	<0.001*
Hypertension	147 (16.7)	44 (7.2)	53 (29.4)	50 (58.1)	<0.001*
Diabetes mellitus	73 (8.3)	19 (3.1)	24 (13.3)	30 (34.9)	<0.001*
Cardiovascular disease	64 (7.3)	18 (2.9)	19 (10.6)	27 (31.4)	<0.001*
Chronic pulmonary disease	47 (5.3)	25 (4.1)	11 (6.1)	11 (12.8)	0.003*
Immunosuppression	36 (4.1)	8 (1.3)	17 (9.4)	11 (12.8)	<0.001*
Chronic renal failure	32 (3.6)	4 (0.7)	11 (6.1)	17 (19.8)	<0.001*
Hospitalization (day) n=183 median (IQR)	9 (5-13)	1.5 (1-ND)	8 (6-12)	10 (5-16.5)	0.018*
Mechanical ventilation n (%)	30 (3.4)	0 (0)	2 (1.1)	28 (32.6)	<0.001*
Exitus n (%)	50 (5.7)	0 (0)	2 (1.1)	48 (55.8)	<0.001*

IQR: Interquartile range
*p-value <0.05, ND: Not defined, because only two patients data are available

Table 2: The analysis of the laboratory parameters

Variables	All n=880	Mild n=614	Moderate n=180	Severe/critical n=86	p-value
PCR CT value median (IQR)	22 (18-25)	22 (19-25)	23 (18-25)	22 (18-25)	0.882
Other laboratory analysis	**All n=534**	**Mild n=313**	**Moderate n=136**	**Severe/critical n=85**	
Lymphocyte (/µL) median (IQR)	1070 (795-1610)	1450 (1075-2010)	1310 (930-1727)	950 (625-1560)	<0.001*
Lymphopenia <800 n (%)	77 (8.8)	28 (8.9)	14 (10.3)	35 (41.2)	<0.001*
CRP (mg/dL) median (IQR)	37 (10-113)	5 (2-11)	16 (6-37)	112 (45-163.5)	<0.001*
CRP >50 n (%)	95 (10.8)	8 (2.6)	29 (21.3)	58 (68.2)	<0.001*
Ferritin (µg/L) median (IQR)	250 (102-650)	70 (26-148)	137 (72.7-301)	484 (171-977)	<0.001*
Ferritin >500 n (%)	54 (6.1)	5 (2.1)	20 (17.5)	29 (49.2)	<0.001*
D-Dimer (mg/L) median (IQR)	0.68 (0.35-1.84)	0.31 (0.2-0.53)	0.5 (0.28-1)	1.17 (0.58-2.93)	<0.001*
D-dimer >1000 n (%)	104 (11.8)	24 (7.8)	35 (25.9)	45 (55.6)	<0.001*

CRP: C-reactive protein, IQR: Interquartile range
*p-value <0.05

gender, comorbidities, length of hospital stays, need for mechanical ventilation, or death (Table 3). In Figure 2, there is no relationship between CT values and disease severity. Figure 3 shows that there is no relationship between CT values and mechanical ventilation support. In Figure 4, there is no relationship between CT values and mortality. Similarly, we found no association between poor prognostic markers and CT values in the laboratory (Table 4).

Discussion

In this study was shown no correlation between CT values and the severity or prognosis of COVID-19. While some studies have found that CT values are correlated with disease severity, disease progression, and mortality (8-10), other studies have found no such relationship (11-13). A report from the Infectious Diseases Society of America (IDSA) and the Association for Molecular Pathology (AMP) noted that CT values are affected by many factors and cannot be generalized (14).

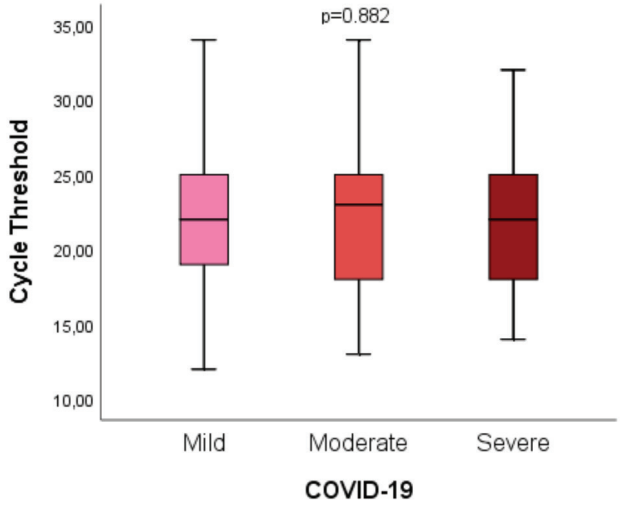

Figure 2: Comparison CT values and COVID-19 severity

CT: Cycle threshold, COVID-19: Coronavirus disease-2019

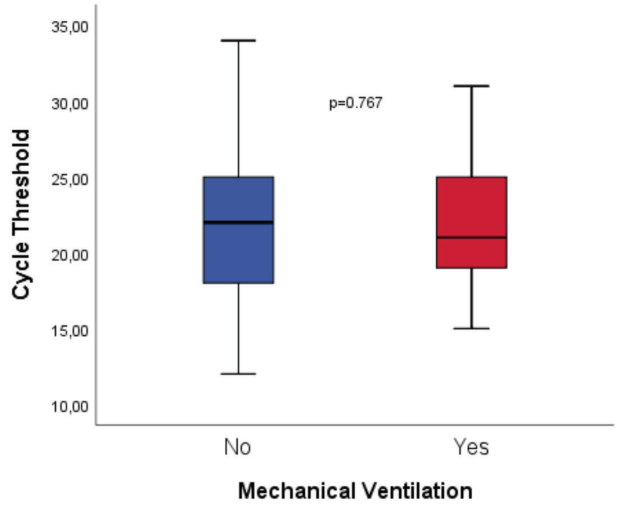

Figure 3: Comparison CT values and mechanical ventilation support

CT: Cycle threshold

Table 3: Evaluation the relationship between CT values and demographic features

Variables	All	Q1 (≤18)	Q2 (19-22)	Q3 (23-25)	Q4 (≥26)	p-value
n (%)	880	225 (25.6)	218 (24.8)	216 (24.5)	221 (25.1)	
Female n (%)	415 (47.2)	111 (49.3)	100 (45.9)	101 (46.8)	103 (46.6)	0.893
Age median (IQR)	63 (50-74)	45 (32-57)	42 (30-53.25)	47 (36-58)	44 (32-57.5)	0.118
Comorbidity n (%)	241 (27.1)	61 (27.1)	60 (27.5)	68 (31.5)	52 (23.5)	0.323
Hypertension	147 (16.7)	36 (16)	33 (15.1)	45 (20.8)	33 (14.9)	0.307
Diabetes mellitus	73 (8.3)	24 (10.7)	17 (7.8)	17 (7.9)	15 (6.8)	0.485
Cardiovascular disease	64 (7.3)	15 (6.7)	16 (7.3)	17 (7.9)	16 (7.2)	0.972
Chronic pulmonary disease	47 (5.3)	10 (4.4)	15 (6.9)	15 (6.9)	7 (3.2)	0.208
Immunosuppression	36 (4.1)	6 (2.7)	9 (4.1)	12 (5.6)	9 (4.1)	0.504
Chronic renal failure	32 (3.6)	6 (2.7)	9 (4.1)	9 (4.2)	8 (3.6)	0.819
Duration of symptoms median (IQR) n=694	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-4)	0.668
At least one poor prognostic marker n (%) n=530	195 (22.2)	47 (38.5)	46 (35.4)	55 (36.4)	47 (37)	0.964
Duration of hospitalization (day) median (IQR) n=183	9 (5-13)	10 (6-15.7)	10.5 (6.7-14)	7 (5-12)	8 (5-13)	0.051
Mechanical ventilation n (%)	30 (3.4)	7 (3.1)	9 (4.1)	7 (3.2)	7 (3.2)	0.504
Mortality n (%)	50 (5.7)	11 (4.9)	15 (6.9)	13 (6)	11 (5)	0.777

IQR: Interquartile range

Molecular tests using respiratory-tract samples are specific to the patient, as are age, immunosuppression, the presence and severity of symptoms, and the duration of the disease. In addition, factors, such as adequacy, place of receipt, transportation, and storage conditions of the samples, can affect CT values. For these reasons, standardization is quite difficult. Currently, there is no quantitative SARS-CoV-2 PCR test approved for immediate use by the United States Food and Drug Administration. Similarly, there is no accepted validation method that provides standardization among the manufacturers who produce the tests and the laboratories that use them. Neither is there any internationally convertible, standard reference material. Rhoads et al. (15) also found significant variation among the CT values of different PCR tests and reported that the target region of the viral gene can vary between 3 and 12 cycles due to the test type and the laboratory. The present study included some standardized factors, such as the same test in the same laboratory environment, interpretation by the same staff, exclusion of lower respiratory-tract samples, and the taking of samples by staff who had the same training. However, the study's patient factors (age, immunosuppression, etc.) could not be uniform. Also, the interval between retrieving and testing samples varied.

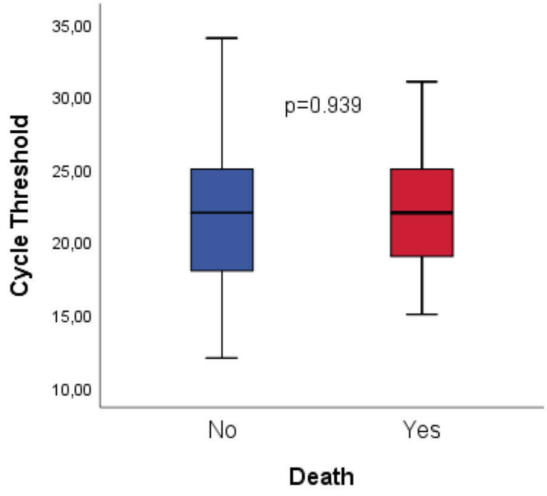

Figure 4: Comparison CT values and mortality
CT: Cycle threshold

The present study found no relationship between CT values and lymphopenia or elevated levels of CRP, D-dimer, or ferritin, which are the poor prognostic markers noted in Turkey's national guidelines. Huang et al. (16) reported lower CT values in critically ill patients than in patients in other groups and viral loads negatively correlated with the portion parameters of the blood routine and lymphocyte subsets. The same study also reported higher viral loads in samples taken from the lower respiratory tract than from the upper respiratory tract. That study included nasal, nasopharyngeal, sputum, bronchoalveolar lavage, and stool samples (16). The appropriateness of including different sample types in the same sample is controversial. Also, the literature reports varying rates (3-30%) of false negativity (17).

Yu et al. (18) found a correlation between symptom day and viral load, with the viral load being higher in patients on earlier symptom days. Our study found no correlation between symptom day and viral load.

Given the dynamic process of COVID-19, there are still many unexplained recommendations. However, unproven recommendations may lead to more aggressive and inappropriate follow-up and treatment approaches. Also, patients may misunderstand such treatment and believe that the disease's progression will be more severe.

On the other hand, we believe that universality of CT values seems impossible, since there is no validated strain for SARS-CoV-2 and it seems unlikely due to newly emerged variant strains.

Study Limitations

Our study has some limitations. Notably, because of its retrospective nature, it could not evaluate sequential SARS-CoV-2 PCR tests. Therefore, it may be more appropriate to evaluate using repeated PCR tests. This could be addressed in a prospective study using a larger number of samples and providing standardization of simultaneous and standardizable parameters. All COVID-19 positive cases were hospitalized for isolation at the beginning of the pandemic. For this reason, we may not have accurately reflected the hospitalization rates.

Table 4: Evaluation CT values and laboratory values relationship

	All	Q1 (<18)	Q2 (19-22)	Q3 (23-25)	Q4 (>26)	p-value
Lymphocyte (/µL) median (IQR)	1070 (795-1610)	1410 (1000-2025)	1360 (947-1795)	1250 (935-1820)	1350 (930-1830)	0.656
CRP (mg/dL) median (IQR)	37 (10-113)	8.5 (3-27)	10 (3-30.7)	9 (4-32.5)	8 (3-33.25)	0.913
Ferritin (µg/L) median (IQR)	250 (102-650)	97 (29.5-196.75)	104 (47-263)	120 (43-255)	96 (38.5-211.75)	0.480
D-Dimer (mg/L) median (IQR)	0.68 (0.35-1.84)	0.35 (0.22-0.75)	0.38 (0.23-0.87)	0.42 (0.21-0.84)	0.44 (0.3-0.76)	0.248

CRP: C-reactive protein, IQR: Interquartile range, CT: Cycle threshold

COVID-19 positive cases from a specific time period were included in our cross-sectional study. According to the global course of COVID-19, the majority of people infected with the virus experience mild to moderate respiratory illness. The unequal distribution of the disease severity groups in our study reflects this. The number of patients requiring mechanical ventilation were low. Finally, because we could not get consecutive samples from each patient, we do not know the effect of progress of CT on outcomes.

Conclusion

Despite being retrospective, ours is one of the few studies showing that there is no correlation between RT-PCR CT values and COVID-19 disease progression, mortality, and laboratory parameters. Although in practice, there is a relative relationship between CT values and viral load, we agree that IDSA and AMP test results cannot be considered quantitative or generalizable because of the many analytical and clinical factors known to affect CT values.

Ethics

Ethics Committee Approval: This study was approved by the Başkent University Institutional Review Board (project no: KA20/145, date: 12.01.2021).

Informed Consent: Retrospective study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: T.Y.Y., H.A., Design: T.Y.Y., Data Collection or Processing: T.Y.Y., N.S., Ç.E., G.D.Y., Analysis or Interpretation: S.A., Literature Search: T.Y.Y., S.A., N.S., Ç.E., G.D.Y., Writing: T.Y.Y., S.A., Ö.K.A., H.A.

Conflict of Interest: The authors declare no conflicts of interest.

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