RESEARCH ARTICLE / ARAŞTIRMA

DOI: 10.4274/mjima.galenos.2023.2023.24
Mediterr J Infect Microb Antimicrob 2023;12:24
Erişim: http://dx.doi.org/10.4274/mjima.galenos.2023.2023.24

RT-PCR Cycle Threshold for Predicting COVID-19-related Cardiac Complications: A Case-control Study

COVID-19 ile İlişkili Kardiyak Komplikasyonları Öngörmek için RT-PCR Döngü Eşiği: Bir Olgu Kontrol Çalışması

Yasemin NADİR[1], Pınar KIRAN[2]

[1]İzmir Provincial Health Directorate, İzmir University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Infectious Diseases and Cinical Microbiology, İzmir, Turkey
[2]Dokuz Eylül University Faculty of Medicine, Department of Public Health, Division of Epidemiology, İzmir, Turkey

Abstract

Introduction: Coronavirus disease-2019 (COVID-19) causes several cardiovascular (CV) complications. The cycle threshold (Ct) value of reverse transcription-polymerase chain reaction is inversely related to the viral load. Thus, it could be used as a predictor of outcomes. We aimed to present the risk factors for developing CV events and to determine whether the Ct value can be used as a predictor of CV events in patients with COVID-19.
Materials and Methods: This case-control study included 296 hospitalized patients with a confirmed COVID-19 infection. The patients were divided into two groups based on the occurrence of CV events: cardiac (n=60) and non-cardiac (n=236). The clinical manifestation, comorbidities history, and laboratory and radiographic findings were compared between the two groups. In order to assess the link between CV complications and Ct values while controlling for confounders, binary logistic regression analysis was used. The receiver operating characteristic (ROC) curve analysis was used for estimating the cut-off Ct value for predicting the occurrence of CV events.
Results: Approximately 50% of the patients were male. The mean age was 60.85±19.57 years. Binary logistic regression analysis demonstrated that a lower Ct value at the time of admission [odds ratio (OR)=0.836, 95% confidence interval (CI): 0.753-0.928, p=0.001], higher troponin level (OR=1.209, 95% CI: 1.050-1.392, p=0.008), smoking history (OR=7.336, 95% CI: 3.34-16.114, p<0.001), advanced age (OR=1.022, 95% CI: 1.001-1.044, p=0.039), and the male sex (OR=2.742, 95% CI: 1.271-5.919, p=0.010) were independent risk factors of CV events in patients with COVID-19. The median Ct for all participants was 24.6 (21-28). The Ct value demonstrated a sensitivity of 72%, specificity of 72%, negative predictive value of 91%, and positive predictive value of 40% for the prediction of CV complications at a cut-off value of 23, according to an ROC curve analysis.
Conclusion: A Ct of <23 at the time of admission for COVID-19 could predict the risk of a CV event. Thus, if patients with risk factors for CV events have a Ct of <23 Ct on admission, they should be evaluated by a competent COVID-19 heart team.
Keywords: COVID-19, cycle threshold, cardiac injury

Öz

Giriş: Koronavirüs hastalığı-2019 (COVID-19) enfeksiyonu çeşitli kardiyovasküler komplikasyonlara neden olabilir. Bilindiği gibi, revers-transkriptaz polimeraz zincir reaksiyonunun (RT-PCR) döngü eşik (Ct) değeri, viral yük ile ters orantılıdır ve bu nedenle COVID-19 prognozu açısından fikir verebilir. Bu çalışmada Ct değerinin COVID-19 hastalarında kardiyovasküler olay gelişiminde bir öngörücü olarak kullanılıp kullanılamayacağını araştırmayı amaçlıyoruz.
Gereç ve Yöntem: Bu çalışma tek merkezde yapılan, retrospektif bir olgu-kontrol çalışmasıdır. Çalışmaya COVID-19 RT-PCR pozitif olan ve hastaneye yatırılmış 296 hasta dahil edildi. Kardiyovasküler olayların oluşumuna göre katılımcılar iki gruba ayrıldı (n=60 kardiyak grup, n=236 kardiyak olmayan grup). İki grup arasında klinik bulgular, kronik hastalık öyküsü, laboratuvar ve radyolojik bulgular karşılaştırıldı. Karıştırıcı değerleri kontrol altında tutarak kardiyovasküler olaylar ile Ct değerleri arasındaki bağlantıyı değerlendirmek için binary lojistik regresyon analizi kullanıldı. Kardiyovasküler olayların oluşumunu gösteren Ct cut-off değerini tahmin etmek için ROC analizi yapıldı.

Cite this article as: Nadir Y, Kıran P. RT-PCR Cycle Threshold for Predicting COVID-19-related Cardiac Complications: A Case-control Study. Mediterr J Infect Microb Antimicrob. 2023;12:24.

Address for Correspondence/Yazışma Adresi: Yasemin NADİR MD, İzmir Provincial Health Directorate, İzmir University of Health Sciences Turkey, İzmir Tepecik Training and Research Hospital, Clinic of Infectious Diseases and Cinical Microbiology, İzmir, Turkey
Phone: +90 505 675 53 38 **E-mail:** dr.yaseminz@gmail.com **ORCID ID:** orcid.org/0000-0003-1346-4579
Received/Geliş Tarihi: 10.05.2023 **Accepted/Kabul Tarihi:** 12.09.2023
Published: 21.09.2023

Bulgular: Hastaların yaklaşık %50'si erkekti. Ortalama yaş 60,85±19,57 idi. Daha önce kronik kalp hastalığı geçirme öyküsü kardiyak grupta istatistiksel olarak daha yüksekti. Ayrıca yapılan binary lojistik regresyon analizi, başvuru sırasındaki düşük Ct değeri, yüksek troponin düzeyi, sigara içme öyküsü, ileri yaş ve erkek cinsiyetin kardiyovasküler olayların ortaya çıkmasıyla yüksek oranda ilişkili olduğunu göstermiştir. Tüm katılımcılar için RT-PCR'nin medyan Ct değeri 24,6 çeyrekler arası aralık (21-28) idi. Ct değeri için 23 cutoff değerinin, ROC eğrisi analizine göre kardiyak problemlerin tahmini için %72 duyarlılık, %72 özgüllük, %91 negatif öngörü değeri ve %40 pozitif öngörü değerine sahip olduğu gösterildi.
Sonuç: Çalışmamız, hastanede yatan COVID-19 hastalarının kabulünde <23 Ct değerinin, gelişen kardiyovasküler olayları öngörebileceğini düşündürmektedir. Sonuç olarak, hastaların kardiyak komplikasyonlar için risk faktörleri varsa ve başvuruda <23 Ct değeri saptanırsa, kardiyovasküler komplikasyon gelişimi açısından dikkatli olunmalıdır.
Anahtar Kelimeler: COVID-19, cycle threshold, kardiyak hasar

Introduction

The Coronavirus disease-2019 (COVID-19) pandemic first broke out in China in December 2019, and it is still ongoing[1]. The gold standard for the diagnosis of COVID-19 is real-time reverse transcription-polymerase chain reaction (RT-PCR)[2]. The cycle threshold (Ct) values are used to measure the amplification in RT-PCR. The Ct value is the number of cycles at which fluorescence of the PCR product is detectable over and above the background signal. Several PCR assays use 40 as the Ct cut-off for the test to be considered positive. The Ct value is inversely related to the viral load[3]. Thus, it can be used as a predictor of the severity and outcomes of COVID-19[4].

Coronavirus disease-2019 causes numerous cardiovascular (CV) complications. Cardiac arrest, myocarditis, myocardial injury, cardiomyopathy, arrhythmias, and heart failure are the most common CV complications[5]. The main mechanism underlying the CV events may be the serious systemic inflammation associated with the COVID-19 infection[6]. Recent studies have demonstrated that COVID-19 infection-associated heart damage is associated with morbidity and mortality[7].

If patients at a high risk of developing CV events can be identified early, the management of COVID-19 could improve. Thus, we needed to identify which patients with COVID-19 developed cardiac events. A study showed thatgender, race, a lower SaO_2/FiO_2 ratio, a higher serum potassium concentration, a lower serum albumin concentration, and the presence of comorbidities were associated with the occurrence of CV events in patients with COVID-19[8]. Several risk assessment systems have been used to predict adverse events in patients with COVID-19. However, cardiac events cannot be predicted before the appearance of symptoms.

Through this study, we aimed to present the risk factors associated with the development of CV events and determine whether the Ct values of RT-PCR on admission can be used as a predictor of CV events in patients with COVID-19.

Materials and Methods

Study Design, Clinical Management, and Data Collection

This was a single-center case-control study conducted in İzmir, Turkey, which included patients with confirmed COVID-19 infections from January 2020 to August 2022. The patients were included in the study if they had a positive Severe acute respiratory syndrome-Coronavirus 2 (SARS-CoV-2) RT-PCR test, were inpatients, and were aged ≥18 years. The patients were divided into two groups: non-cardiac (those who did not experience any cardiac events) and cardiac (those who experienced a cardiac event during the COVID-19 infection). A sample size of 280 was determined using G-Power (version 3.1.9.7; Düsseldorf, Germany), with 99% power and 5% type-1 error[9].

In this study, the primary outcome variable of interest was any CV event. CV events were defined as any cardiac complications (heart failure, myocardial injury, cardiomyopathy, myocarditis, arrhythmias, pulmonary embolism, deep vein thrombosis, and cardiac arrest) that were diagnosed at the time of hospitalization for COVID-19 or within one month after discharge.

Demographic/laboratory data, comorbidities history, clinical manifestations, laboratory values, and Ct results were obtained from the electronic medical records of the participants.

The upper limits of all biochemical test parameters were based on the reference limits that were determined by the hospital's biochemistry laboratory. The computed tomography (CT) results were classified according to the "CT severity index": mild (Score 1), <5%; moderate [Score 2 (5-25%) and Score 3 (26-50%)]; and severe [Score 4 (51-75%) and Score 5 (>75%)][5].

The other outcome that was studied was the Ct value of the patient's SARS-CoV-2 RT-PCR at the time of admission. The patients were followed up according to the Turkish Ministry of Health's COVID-19 Guidelines. This study was approved by the Institutional Review Board and the Research and Ethics Committee of the Bozyaka Training and Research Hospital (no: 2022/135; date: 14.09.2022).

SARS-CoV-2 Detection Test

We included patients with a positive RT-PCR test using nasopharyngeal specimens. The SphaeraMag® DNA/RNA Isolation Kit was Austria, Thalgau, Procomcure Biotech) was used for isolating nucleic acids from the nasopharyngeal swabs with an automated nucleic acid extraction system. Subsequently, the PhoenixDx® SARS-CoV-2 Detection Kit was used for the RT-PCR. This kit quantifies the SARS-CoV-2 virus by targeting the open reading frame 1ab and nucleocapsid protein genes. We used the COBAS LightCycler® 480 system (Roche) to determine SARS-CoV-2. The typical S-shaped amplification curves of the FAM and ROX fluorescence channels and Ct values <40 were defined as a positive test result. The Ct levels were recorded using this technique in hospitalized individuals with positive RT-PCR results.

Statistical Analysis

The continuous variables were described as means±standard deviations or medians and interquartile ranges according to the Kolmogorov-Smirnov test. The categorical variables were described using numbers (n) and percentage (%) distributions. The parametric, non-parametric, and categorical data of the cardiac and non-cardiac groups were compared using the Student's t-test, Mann-Whitney U test, and chi-square test, respectively. A p value of <0.05 was considered statistically significant. Variables that were statistically significant in the univariate analysis were included in multivariate analysis to identify independent risk factors for CV events. The odds ratios (OR) and 95% confidence intervals (CI) were estimated using binary logistic regression models[6]. The model fit was evaluated using the Hosmer-Lemeshow goodness of fit statistics. The receiver operating characteristic (ROC) curve analysis was used to estimate the Ct cut-off level for predicting the occurrence of CV events. The area under the ROC curve (AUC) was calculated to evaluate the diagnostic value of the logistic regression model and the Ct value. All statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp).

Results

Patient Characteristics

A total of 296 patients with COVID-19 were included in the study. A total of 60 patients met our inclusion criteria in the cardiac group, and 236 matched controls were included in the non-cardiac group. The baseline characteristics of the patients with COVID-19 in the cardiac and non-cardiac groups are summarized (Table 1). Approximately 50% of the patients were male. The mean age of the participants was 60.85±19.57 years. The most commonly occurring comorbidities were diabetes mellitus (52%), hypertension (46%), and chronic lung disease (22%), respectively. A total of 36 patients (12%) had a history of cardiac disease, and 22 of these had coronary artery disease. A history of smoking was reported in 66 (22%) patients. The mean body mass index (BMI) was 28.2±5.6 kg/m^2. A total of 60 patients developed cardiac complications.

Clinical Outcome

This study included patients hospitalized with COVID-19. Of the included patients, 93 (31%) required mechanical ventilation, 85 (28.7%) required noninvasive ventilation, and 118 (40.3%) required nasal cannula oxygen or did not require oxygen. Most of the patients presented with fever, dyspnea, and myalgia. The most common cardiac complications were cardiac injury (n=37, 61.6%), heart failure (n=13, 21.6%), arrhythmia (n=4, 6.6%), and myocarditis (n=3, 5%).

There were no differences in the clinical manifestations or comorbidities between the cardiac and non-cardiac groups. However, a prior history of chronic heart disease was statistically higher in the cardiac group than in the non-cardiac group (p<0.001). The cardiac group had more males, older patients, and a positive smoking history than the non-cardiac group (p=0.018, p=0.001, and p<0.001, respectively). However, there was no difference in the need for invasive mechanical ventilation and the patient's BMI between the two groups (p=0.791).

The laboratory and radiographic findings of the patients in the cardiac and non-cardiac groups at the time of admission are summarized in Table 2. There were no differences in the laboratory values between the two group, except for the troponin and Ct values (p<0.001 and p<0.001, respectively), which was statistically higher in the cardiac group than in the non-cardiac group (p<0.001). The median Ct value was 24.6 (21-28) for all patients, 21.1 (20-23.7) in the cardiac group, and 25.6 (22-29) in the non-cardiac group.

Binary logistics regression analysis revealed that a lower Ct value on admission (OR=0.836, 95% CI: 0.753-0.928, p=0.001), higher troponin levels (OR=1.209, 95% CI: 1.050-1.392, p=0.008), smoking history (OR=7.336, 95% CI: 3.34-16.114, p<0.001), advanced age (OR=1.022, 95% CI: 1.001-1.044, p=0.039) and male sex (OR=2.742, 95% CI: 1.271-5.919, p=0.010) were independent risk factors for CV events in patients with COVID-19 (Table 3).

The Ct value demonstrated a sensitivity of 72%, specificity of 72%, negative predictive value of 91% and positive predictive value of 40% for the predicting cardiac complications at a cut-off value of 22.95, according to the ROC curve analysis. The AUC was 0.764 (95% CI: 0.7-0.828; p<0.001). Evaluation of the diagnostic value of the logistic regression model with the

ROC curve analysis, revealed an AUC of 0.903 (95% CI: 0.864-0.942; p<0.001) (Figure 1). There was a statistically significant relationship between a lower Ct level at the time of admission and the occurrence of CV events.

Discussion

Coronavirus disease-2019 continues to affect the whole world and causes several complications. CV events may be the most important complications because there is reportedly an increase in the mortality rate when the COVID-19 infection is associated with a CV event. The main mechanisms underlying CV events in COVID-19 infections may be the high inflammatory burden and microvascular dysfunction[10]. The SARS-CoV-2 virus reportedly causes microvascular dysfunction via the ACE2 transmembrane protein[11].

The CV event could manifest as several clinical characteristics, such as myocardial injury (acute coronary syndrome and acute myocardial infarction), myocarditis, cardiac arrhythmia, cardiac arrest, venous thromboembolic disease, and heart failure[12]. In this study the most common manifestation was myocardial injury.

Considering the increase in mortality and morbidity caused, it would be very valuable to predict CV complications in patients with COVID-19. Thus, several studies have sought

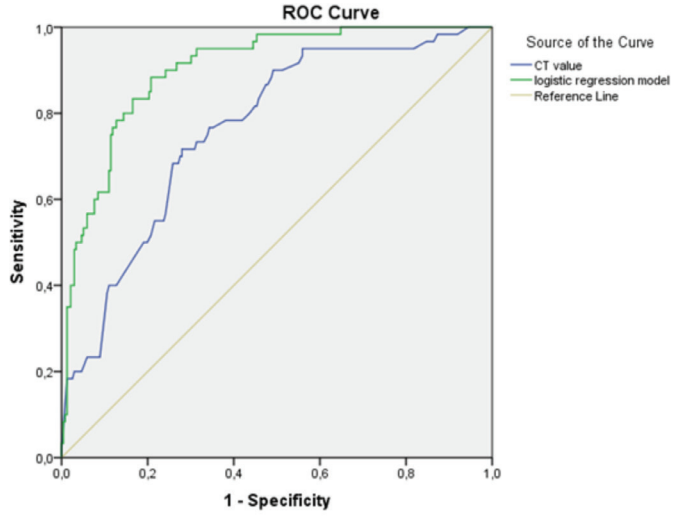

Figure 1. ROC curve analysis of the logistic regression model for the performance of Ct value in predicting the cardiac complication

ROC: Receiver operating characteristic, Ct: Cycle threshold

Table 1. Baseline characteristics of patients with COVID-19 who did and did not develop CV events

Variables	Patients who did not developed cardiac complications (n=236)	Patients who developed cardiac complications (n=60)	p value
Age, mean±SD	59±20.61	68±12.37	**0.001**
Sex, n (%)			
Female	127 (53.8)	22 (36.7)	**0.018**
Male	109 (46.2)	38 (63.3)	
BMI, mean±SD	28.3 (5)	28.1 (6)	0.791
Clinical history, n (%)			
Chronic heart disease	18 (7.6)	18 (30)	**<0.001**
Cancer	6 (2.5)	2 (3.3)	0.736
Diabetes mellitus	133 (56)	21 (35)	0.157
Hypertension	106 (45)	30 (50)	0.480
Chronic lung diseases	46 (19.4)	18 (30)	0.077
Chronic kidney disease	5 (2)	2 (3.3)	0.580
Smoking history	30 (12.7)	36 (60)	**<0.001**
Cerebrovascular disease	2 (0.8)	1 (1.7)	0.296
Clinical manifestation, n (%)			
Fever	102 (43.2)	25 (41.7)	0.828
Cough	21 (8.9)	3 (5)	0.432
Dyspnea	82 (34.7)	20 (33.3)	0.837
Chest pain	18 (7.6)	5 (8.3)	0.855
Myalgia	52 (22)	20 (33.3)	0.069
Headache	14 (5.9)	2 (3.3)	0.426

Data are presented as mean±SD or n (%). The p values were calculated using the Student's t-test, chi-square test, or Fisher's exact test, as appropriate.

COVID-19: Coronavirus disease-2019, CV: Cardiovascular, SD: Standard deviation, BMI: Body mass index

to identify the risk factors of CV events among patients with COVID-19. One study attempted to identify the risk factors using with Framingham risk scores[13,14]. In some studies, older age and presence of comorbidities (hypertension, coronary heart disease, and chronic obstructive pulmonary disease) were found to be predictors of CV events[15,16]. Furthermore, a binary multiple logistics analysis demonstrated that the male sex, race, and CV comorbidities were highly associated with the occurrence of CV events in patients with COVID-19[17]. A retrospective cohort analysis demonstrated that higher potassium levels and lower albumin levels were associated with CV events in patients with COVID-19[18]. However, in some studies, there were no statistically significant differences in the laboratory findings between patients did and did not develop CV events[19].

Our binary logistics regression analysis revealed that a higher troponin level, presence of smoking history, advanced age, and the male sex were highly associated with the occurrence of CV events in patients with COVID-19. These findings are similar to those of several recent studies. However, they are not enough to determine the risk of developing CV events in patients with COVID-19.

Table 2. Laboratory and radiographic findings of patients with COVID-19 who did and did not develop CV events at the time of admission

Variables	Patients who developed cardiac complications (n=236)	Patients who did not develop cardiac complications (n=60)	p value
Laboratory findings			
WBC, 10^9/l	7.21 (6.25-9.42)	7.65 (6.95-9.78)	0.421
Lymphocytes, 10^9/l	1.32 (0.7-1.7)	1.48 (0.82-1.94)	0.582
CRP, mg/l	27 (15-130)	23 (22-102)	0.722
D-dimer, mg/l	480 (280-880)	510 (320-700)	0.813
Ferritin, ng/ml	182.5 (448.75)	121 (273.75)	0.134
Troponin ng/ml	0.1 (0.1-0.2)	1.5 (0.1-5.05)	**<0.001**
Urea mg/dl	35 (22-62)	33 (25-45)	0.340
Creatinine mg/dl	0.9 (0.6-1.5)	0.9 (0.8-1.2)	0.877
ALT (U/l)	24 (15-44.7)	26 (17.2-44.2)	0.582
AST (U/l)	27.5 (20-51)	25 (20-46.5)	0.984
Ct value	25.6 (22-29)	21.1 (20-23.7)	**<0.001**
Chest CT			
Normal	50 (21.2)	16 (26.6)	0.528
Mild	64 (27.1)	18 (30)	
Moderate	49 (20.8)	13 (21.7)	
Severe	73 (30.9)	13 (21.7)	

Data are presented as median (IQR) or n (%).

The p values were calculated using the Mann-Whitney U test, chi-square test, or Fisher's exact test, as appropriate.

COVID-19: Coronavirus disease-2019, CV: Cardiovascular, WBC: White blood cell, CRP: C-reactive protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Ct: Cycle threshold, CT: Computed tomography

Table 3. Multivariate logistic regression analysis of the factors associated with CV events

Factor	B	SE	Wald	OR (95% CI)	p value
Ct value	−0.18	0.053	11.318	0.836 (0.753-0.928)	**0.001**
Troponin level	0.19	0.072	7	1.209 (1.050-1.392)	**0.008**
Smoking history	1.993	0.401	26.64	7.336 (3.34-16.114)	**<0.001**
Age	0.022	0.011	4.273	1.022 (1.001-1.044)	**0.039**
Male sex	1.009	0.392	6.607	2.742 (1.271-5.919)	**0.010**
History of chronic heart disease	0.702	0.506	1.923	2.017 (0.748-5.436)	0.166

Nagelkerke R2: 0.479, Hosmer-Lemeshow test: p=0.749, −2 log likelihood: 191.2, Omnibus test: p<0001

CV: Cardiovascular, SE: Standard error, OR: Odds ratio, CI: Confidence interval, Ct: Cycle threshold

The Ct depicts the number of cycles at which fluorescence of the PCR product is detectable. The Ct value is inversely related to the viral load[20]. Bullard et al.[21] suggested that the SARS-CoV-2 infectivity reduces when the Ct value becomes >24. However, some studies have demonstrated that the Ct values are not associated with the COVID-19 severity[22-24]. The Ct value reportedly changes according to when the patients was infected and when the RT-PCR was performed. This might affect the analysis. A meta-analysis of seven studies showed no significant difference in mean Ct values between hospitalized and non-hospitalized patients. However, among the hospitalized patients, those with Ct values <25 had a higher risk of developing a more severe disease and mortality than patients with Ct values >30[25]. Therefore, the Ct value appears to be more reasonable in terms of demonstrating the severity of the infection among hospitalized patients with COVID-19.

Recent studies have suggested that the severity of COVID-19 pneumonia could be a risk factor for the development of cardiac symptoms[14]. However, several studies have attempted to predict the development of cardiac complications using indicators of the severity of COVID-19 infection. Furthermore, there are no studies assessing the relationship between Ct values and the occurrence of cardiac complications. Thus, this study was conducted to determine whether the Ct values can be used as a predictor of CV events. Our multiple logistics regression analysis revealed that a lower Ct value at the time of admission was highly associated with the occurrence of CV events in patients hospitalized with COVID-19. The cut-off Ct value for developing CV events was 23 in our study. This result is supported by those of some other studies, where the cut-off value for the severity of COVID-19 infection was similar[25].

Study Limitations

The most important limitation of this study is that it was a retrospective study. The patients characteristics and laboratory and radiographic findings were extracted from electronic medical records. This may have caused an information bias. Another limitation of the study is that only hospitalized patients were included. Furthermore, all the CV events may not have been diagnosed; the outpatients may have not been diagnosed with CV disease. Thus, further studies are required for this topic especially including larger populations.

Conclusion

CV complications could occur in the presence of risk factors (male sex, advanced age, and smoking history) and a Ct of <23 at the time of admission in hospitalized patients with COVID-19. If these patients are evaluated by a competent COVID-19 heart team, CV events could be detected early, optimizing the management of the COVID-19 outcomes.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Review Board and the Research and Ethics Committee of the Bozyaka Training and Research Hospital (no: 2022/135; date: 14.09.2022).

Informed Consent: The author has obtained informed consent from the patient(s).

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.N., Concept: Y.N., Design: Y.N., P.K., Data Collection or Processing: Y.N., Analysis or Interpretation: Y.N., P.K., Literature Search: Y.N., P.K., Writing: Y.N.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. J Med Virol. 2020;92:401-2.
2. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, Han Y, Hu B, Hu F, Li BH, Li YR, Liang K, Lin LK, Luo LS, Ma J, Ma LL, Peng ZY, Pan YB, Pan ZY, Ren XQ, Sun HM, Wang Y, Wang YY, Weng H, Wei CJ, Wu DF, Xia J, Xiong Y, Xu HB, Yao XM, Yuan YF, Ye TS, Zhang XC, Zhang YW, Zhang YG, Zhang HM, Zhao Y, Zhao MJ, Zi H, Zeng XT, Wang YY, Wang XH; for the Zhongnan Hospital of Wuhan University Novel Coronavirus Management and Research Team, Evidence-Based Medicine Chapter of China International Exchange and Promotive Association for Medical and Health Care (CPAM). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020;7:4.
3. Tom MR, Mina MJ. To Interpret the SARS-CoV-2 Test, Consider the Cycle Threshold Value. Clin Infect Dis. 2020;71:2252-4.
4. Garg K, Sharma K, Gupta A, Chopra V. Association of cycle threshold values of CBNAAT with severity and outcome in COVID-19. Monaldi Arch Chest Dis. 2021;91.
5. Magadum A, Kishore R. Cardiovascular Manifestations of COVID-19 Infection. Cells. 2020;9:2508.
6. Liu F, Liu F, Wang L. COVID-19 and cardiovascular diseases. J Mol Cell Biol. 2021;13:161-7.
7. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020;323:1061-9.
8. Xu Q, Samanapally H, Nathala P, Salunkhe V, Furmanek S, Cahill MN, McGuffin T, Mohammad T, Marsili B, Petrey J, Carrico R, Ramirez J, Akca O, Clifford SP, Pahwa S, Roser L, Kong M, Huang J; Center of Excellence for Research in Infectious Diseases (CERID) Coronavirus Study Group on behalf of the COVID-19 CardioVascular Research Group (COVID-CVRG). Outcomes and Risk Factors for Cardiovascular Events in Hospitalized COVID-19 Patients. J Cardiothorac Vasc Anesth. 2021;35:3581-93.

9. Choudhuri J, Carter J, Nelson R, Skalina K, Osterbur-Badhey M, Johnston A, Goldstein D, Paroder M, Szymanski J. SARS-CoV-2 PCR cycle threshold at hospital admission associated with patient mortality. PLoS One. 2020;15:e0244777.

10. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential Effects of Coronaviruses on the Cardiovascular System: A Review. JAMA Cardiol. 2020;5:831-40.

11. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, Madhur MS, Tomaszewski M, Maffia P, D'Acquisto F, Nicklin SA, Marian AJ, Nosalski R, Murray EC, Guzik B, Berry C, Touyz RM, Kreutz R, Wang DW, Bhella D, Sagliocco O, Crea F, Thomson EC, McInnes IB. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. Cardiovasc Res. 2020;116:1666-87.

12. Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, Brown TS, Der Nigoghossian C, Zidar DA, Haythe J, Brodie D, Beckman JA, Kirtane AJ, Stone GW, Krumholz HM, Parikh SA. Cardiovascular Considerations for Patients, Health Care Workers, and Health Systems During the COVID-19 Pandemic. J Am Coll Cardiol. 2020;75:2352-71.

13. Alshaikh MK, Alotair H, Alnajjar F, Sharaf H, Alhafi B, Alashgar L, Aljuaid M. Cardiovascular Risk Factors Among Patients Infected with COVID-19 in Saudi Arabia. Vasc Health Risk Manag. 2021;17:161-8.

14. Xu H, Hou K, Xu R, Li Z, Fu H, Wen L, Xie L, Liu H, Selvanayagam JB, Zhang N, Yang Z, Yang M, Guo Y. Clinical Characteristics and Risk Factors of Cardiac Involvement in COVID-19. J Am Heart Assoc. 2020;9:e016807.

15. Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, Cao S, Liu X, Xiang Y, Zhao Q, Huang H, Yang B, Huang C. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. Eur Heart J. 2020;41:2070-9.

16. Al-Wahaibi K, Al-Wahshi Y, Mohamed Elfadil O. Myocardial Injury Is Associated with Higher Morbidity and Mortality in Patients with 2019 Novel Coronavirus Disease (COVID-19). SN Compr Clin Med. 2020;2:2514-20.

17. Xie J, Covassin N, Fan Z, Singh P, Gao W, Li G, Kara T, Somers VK. Association Between Hypoxemia and Mortality in Patients With COVID-19. Mayo Clin Proc. 2020;95:1138-47.

18. Xu Q, Samanapally H, Nathala P, Salunkhe V, Furmanek S, Cahill MN, McGuffin T, Mohammad T, Marsili B, Petrey J, Carrico R, Ramirez J, Akca O, Clifford SP, Pahwa S, Roser L, Kong M, Huang J; Center of Excellence for Research in Infectious Diseases (CERID) Coronavirus Study Group on behalf of the COVID-19 CardioVascular Research Group (COVID-CVRG). Outcomes and Risk Factors for Cardiovascular Events in Hospitalized COVID-19 Patients. J Cardiothorac Vasc Anesth. 2021;35:3581-93.

19. Cuomo G, Puzzolante C, Iadisernia V, Santoro A, Menozzi M, Carli F, Digaetano M, Orlando G, Franceschini E, Bedini A, Meschiari M, Manzini L, Corradi L, Milic J, Borghi V, Brugioni L, Pietrangelo A, Clini E, Girardis M, Guaraldi G, Mussini C. Development of post-COVID-19 cardiovascular events: an analysis of clinical features and risk factors from a single hospital retrospective study. Infez Med. 2021;29:538-49.

20. Al Bayat S, Mundodan J, Hasnain S, Sallam M, Khogali H, Ali D, Alateeg S, Osama M, Elberdiny A, Al-Romaihi H, Al-Thani MHJ. Can the cycle threshold (Ct) value of RT-PCR test for SARS CoV2 predict infectivity among close contacts? J Infect Public Health. 2021;14:1201-5.

21. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Doan K, Bastien N, Li Y, Van Caeseele PG, Poliquin G. Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. Clin Infect Dis. 2020;71:2663-6.

22. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020;26:672-5.

23. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, Yip CC, Cai JP, Chan JM, Chik TS, Lau DP, Choi CY, Chen LL, Chan WM, Chan KH, Ip JD, Ng AC, Poon RW, Luo CT, Cheng VC, Chan JF, Hung IF, Chen Z, Chen H, Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;20:565-74.

24. Lee S, Kim T, Lee E, Lee C, Kim H, Rhee H, Park SY, Son HJ, Yu S, Park JW, Choo EJ, Park S, Loeb M, Kim TH. Clinical Course and Molecular Viral Shedding Among Asymptomatic and Symptomatic Patients With SARS-CoV-2 Infection in a Community Treatment Center in the Republic of Korea. JAMA Intern Med. 2020;180:1447-52.

25. Shah VP, Farah WH, Hill JC, Hassett LC, Binnicker MJ, Yao JD, Murad MH. Association Between SARS-CoV-2 Cycle Threshold Values and Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. Open Forum Infect Dis. 2021;8:ofab453.