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Coronavirus Pandemic

Association of SARS-CoV-2 cycle threshold (Ct) values with clinical course and serum biomarkers in COVID-19 patients

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Abstract

Introduction: Our knowledge has gaps regarding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication levels and its association to severity of Coronavirus disease 2019 (COVID-19). The aim of this study was to investigate the association of SARS-CoV-2 viral load with disease severity and serum biomarkers in COVID-19 patients.

Methodology: Viral load was determined via cycle threshold (Ct) values of SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in 214 adult patients. Ct values were compared with clinical severity, biochemical and hematological biomarkers.

Results: Clinical course of the disease was mild (49.1%), moderate (40.2%), and severe (10.7%). Median Ct value was 28.2 (IQR: 22.2–33.8) during the first week of the disease. Ct values were lower within five days after symptom onset [lowest Ct value on the third day (median: 24, IQR: 20.6–32.3)], but they increased significantly during the second and third weeks. No association was detected between admission Ct values and disease severity. Gender, age, co-morbidity, and mortality did not differ significantly in patients with low (≤ 25) and high (> 25) Ct values. White blood cell, neutrophil, platelet, and especially lymphocyte counts, were significantly lower in patients with low Ct values.

Conclusions: No definitive/clear correlation between SARS-CoV-2 viral load and severity and mortality was found in the studied COVID-19 patients. However, neutrophil, platelet, and especially lymphocyte count were significantly lower in patients with a high viral load.

Key words: COVID-19, SARS-CoV-2, Ct value, severity, lymphocyte.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a respiratory tract pathogen that has caused the coronavirus disease 2019 (COVID-19) pandemic which affected the entire world within a short time [1]. Symptomatic patients often show respiratory symptoms, but the clinical spectrum can be very heterogeneous [2,3]. Causes of severe disease and mortality in SARS-CoV-2 infections and their predictability in the early period of COVID-19 have been argued but still remain unclear. The different clinical courses have been investigated in various studies; patients with comorbidities, such as hypertension, cardiovascular disease, advanced age, diabetes, and male gender, are at risk of serious COVID-19 [4-6]. By contrast, discussions are ongoing on the effects of viral load on the clinical course of COVID-19 patients. The effect of viral load on the course of the disease is not clear yet.

Real-time reverse transcription-polymerase chain reaction (RT-PCR) test is the molecular method generally applied for COVID-19 diagnosis. Real-time RT-PCR cycle threshold (Ct) values represent the number of amplification cycles and are inversely related to the viral load [7]. Although semi-quantitative, Ct values can be used to predict viral load. The viral load-related dynamics may be indicative for the prognosis of the disease. The aim of this study was to investigate the association between the viral load determined via real-time RT-PCR Ct values with the

clinical course of the disease and serum biochemical/hematological biomarkers.

Methodology

Study design

This was a retrospective study of 214 patients (age ≥ 18 years) with laboratory confirmed COVID-19 who were followed up at the Uludag University Hospital from April 1, 2020 to May 12, 2020. Pediatric patients and patients who had negative results of SARS-CoV-2 real-time RT-PCR test were excluded from the study. The study protocol was approved by the Institutional Ethics Committee of Uludag University Faculty of Medicine (No:2020-8/39).

Sample collection and laboratory data

Nasopharyngeal specimens were collected from each patient at admission, and viral RNA was extracted with Bio-speedy1 viral nucleic acid transport medium (vNAT) (Bioexen Ltd., Istanbul, Turkey). Real-time RT-PCR was performed with a Bio-speedy1 COVID-19 qPCR detection kit (Bioexen, Istanbul, Turkey) targeting a SARS-CoV-2-specific RNA-dependent RNA polymerase (RdRp) gene fragment in a CFX96 Touch Real-Time PCR Detection System (BIO-RAD, Dubai, United Arab Emirates) following the manufacturer's recommended protocol. The RT-PCR mix included an extraction and sample quality control targeting the human RNase P gene. A positive and a negative control were included in each run to generate a valid result. Bio-Speedy RT-qPCR SARS-CoV-2 kit has been approved by the FDA (US Food and Drug Administration) and added to the EUA (Emergency Use Approval) list, so it was the first kit to receive World Health Organization (WHO) EUA from Turkey for COVID-19 diagnosis. A Ct value of < 40 was defined as a positive result. We categorized patients according to Ct values as follows: ≤ 25 as a high viral load, and Ct values > 25 as a low viral load based on previously reported studies [7,8].

Data collection

Data on clinical and laboratory characteristics, treatments, and outcomes were obtained from the hospital's electronic medical records using data collection forms. Clinical data included demographic characteristics, comorbidities, date of symptom onset, symptoms and signs, radiologic findings, and application of antiviral/antibacterial treatment agents. Disease severity was classified as mild, moderate, and severe COVID-19 according to the WHO definitions [9]. Comorbidities including hypertension, immunosuppressive condition, diabetes mellitus, malignancy, cardiovascular, lung, renal, liver, and autoimmune diseases were evaluated.

Chest computed tomography (CT) examinations were performed in 207 of the patients during various periods of the disease. The typical radiographic features in COVID-19 patients included ground-glass opacities, multifocal patchy consolidation, and/or interstitial changes with a peripheral distribution [10].

Biochemical/hematological findings were evaluated at admission (1–3 days) to hospital. Routine hematological tests (including white blood cell count [WBC], leukocyte subtypes, and platelet counts) were measured with an Abbott Cell-Dyn Ruby system multi-parameter automated hematology analyzer (Abbott Laboratories, Illinois, USA). The automatic immunoanalyzer and a chemistry analyzer (Architect İ2000SR and Architect C8000, Abbott Laboratories, Illinois, USA) were used to measure inflammatory markers and biochemical parameters. D-dimer levels were measured with a Siemens BCS XP System (Erlangen, Germany).

Statistical analysis

The consistency of continuous variables to normal distribution was examined with the Shapiro-Wilk test. According to the normality test result, continuous variables are expressed with median, interquartile range (IQR), minimum and maximum values. Categorical variables were presented as n (%). Statistical comparisons between the mild, moderate and severe groups were evaluated by analysis of Kruskal-Wallis tests. Subgroup analyses were also performed with Dunn-Bonferroni posthoc method after the Kruskal-Wallis tests. Categorical variables were compared using the Fisher-Freeman-Halton, Chi-square, and Fisher's exact tests. Statistical analyses were performed by using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered statistically significant for all comparisons.

Results

This study included 214 adult patients with a positive SARS-CoV-2 real-time RT-PCR result. All patients were in the first week of their illness. The median of the sampling time from the onset of symptoms was two days (min-max: 1–7) and 75% of the samples were taken during the first three days of symptoms. The clinical course of the patients was classified as mild (n = 105; 49.1%), moderate (n = 86; 40.2%), or severe (n = 23; 10.7%) according to the WHO guidelines. The demographic and clinical

information of the patients is shown in Table 1. The median age in the cohort was 41.5 years. Age was significantly higher ($p < 0.001$) in the severe disease group. Healthcare workers accounted for approximately 25% of the cohort, and their disease was mostly mild/moderate (94.7%), with no deaths recorded. Overall, 63.6% of the patients in this study were hospitalized and 5.6% were admitted to intensive care units. The presence of comorbidities was significantly associated with severe illness (between $p = 0.001$ to $p = 0.04$). Hypertension (14%), diabetes mellitus (8.9%), and cardiovascular disease (8.9%) were the most common underlying comorbidities.

The clinical symptoms of patients were cough (61.7%), fatigue/myalgia (58.9%), fever (33.6%), sore throat (27.6%), dyspnea (18.7%), diarrhea (17.8%), headache (16.8%), and tachypnea (6.5%); these were more common in severe disease (between $p < 0.001$ to $p = 0.018$).

The therapeutic agents used for treatment were antivirals (47.2%; favipravir 22%, oseltamivir 25.2%), antibacterials (38.3%), hydroxychloroquine (93%), anticoagulants (10.7%), and immune plasma (3.5%). The frequency of use of these agents was higher in the severe disease group ($p < 0.001$). After the onset of symptoms, the 14-day mortality was 2.8% (n = 6), while 30-day mortality was 5.1% (n = 11). The highest mortality (47.8%; 9.4 times higher than the whole study group) was detected in the severe disease group.

Median SARS-CoV-2 RT-PCR Ct values of the 214 adult patients was 28.2 (IQR: 22.2–33.8) at admission. Additionally, we detected a dynamic change of Ct value during the course of the infection. The lowest median Ct value (highest viral load) was observed on the third day [median (IQR): 24 [20.6–32.3)], and no significant differences were detected during the first five days following symptom onset ($p > 0.05$). However, Ct values started to increase on the sixth day after the onset of symptoms and were significantly higher on the seventh day than on the first, second and third days ($p = 0.039$, 0.031, 0.013, respectively) (Table 2).

During the second (n = 117), third (n = 90), and fourth week (n = 64), patients were re-tested and the percentage of RT-PCR positivity decreased significantly over time. Median Ct values were significantly higher during the second and third weeks than in the first week ($p < 0.01$). No positivity was found in patients (n = 64) tested on the fourth week (Table 2).

The comparison of Ct values in patients with mild, moderate, and severe COVID 19 is given in Table 3. Since no significant difference was detected between Ct

Table 1. Demographic and clinical characteristics of patients with mild, moderate and severe disease.

Variable	Total n = 214	Disease severity			*p*
		Mild n = 105 (49.1%)	Moderate n = 86 (40.2%)	Severe n = 23 (10.7%)	
Demographics and social characteristics					
Age median (IQR)	41.5 (30.0–52.0)	35.0 (26.5–44.0)	45.5 (37.0–54.3)	57.0 (47.0–67.0)	< 0.001
Male (n; %)	115 (53.7)	56 (53.3)	42 (48.8)	17 (73.9)	0.1
Outpatient (n; %)	78 (36.4)	67 (63.8)	11 (12.8)	0	< 0.001
Hospitalized (n; %)	136 (63.6)	38 (36.2)	75 (87.2)	23 (100)	< 0.001
Healthcare workers (n; %)	57 (26.6)	40 (38.1)	14 (16.3)	3 (13.0)	< 0.001
Comorbidities (n; %)					
HT	30 (14.0)	10 (9.5)	14 (16.3)	6 (26.1)	0.04
CVD	19 (8.9)	6 (5.7)	6 (7.0)	7 (30.4)	0.001
DM	19 (8.9)	3 (2.9)	11 (12.8)	5 (21.7)	0.004
CPD	19 (8.9)	11 (10.5)	8 (9.3)	0	0.3
Immunocompromised	10 (4.7)	2 (1.9)	4 (4.7)	4 (17.4)	0.006
Malignancy	8 (3.7)	3 (2.9)	1 (1.2)	4 (17.4)	0.001
Other*	12 (5.6)	6 (5.7)	1 (1.2)	5 (21.7)	0.001
Disease severity					
SpO_2 on room air, med.(min-max)	98 (70-99)	98 (93-99)	97 (91-99)	85 (70-90)	< 0.001
Pulmonary infiltrates					
Bilateral** (n;%)	68 (32.9)	0	47 (54.7)	21 (91.3)	< 0.001
Unilateral/fokal** (n; %)	34 (16.4)	0	34 (39.5)	0	< 0.001
Ventilation or O_2 support					
Non-invasive (n; %)	17 (7.9)	0	2 (2.3)	15 (65.2)	< 0.001
Invasive (n; %)	9 (4.2)	0	0	9 (39.1)	< 0.001
Mortality 14th day (n; %)	6 (2.8)	0	0	6 (26.1)	< 0.001
Mortality 30th day (n; %)	11 (5.1)	0	0	11 (47.8)	< 0.001

**According to the findings of lung computed tomography (n = 204). IQR: interquartile range; HT: Hypertension; CVD: Cardiovascular disease; DM: Diabetes mellitus; CPD: Chronic pulmonary disease (Asthma and obstructive disease). Renal, liver, neurological, and autoimmune diseases.

Table 2. Real time RT-PCR Ct values according to the onset of symptoms.

	Time for symptoms to start before hospital admission	n* (%)**	Median Ct values (IQR)
Baseline Ct values at admission (n = 214)	1 day	70 (100)	27.0 (20.8–33.0)
	2 days	47 (100)	26.5 (22.5–31.6)
	3 days	36 (100)	24.0 (20.6–32.3)
	4 days	17 (100)	28.9 (22.6–35.9)
	5 days	11 (100)	26.8 (25.2–35.5)
	6 days	22 (100)	31.7 (27.4–36.1)
	7 days	11 (100)	33.7 (31.4–39.1)
Ct values of repeated positive during the follow-up of patients	2 weeks	117 (17.1)	33.0 (28.5–34.9)
	3 weeks	90 (7.8)	36.7 (31.2–38.3)
	4 weeks	64 (0.0)	None

Ct: cycle threshold; IQR: interquartile range. *n: number of patients tested; **Percentage of patients with positive tests.

values in the first five days after symptom onset, the median Ct values for the first five days were included together for further analysis. Similarly, the sixth and seventh-day values were also evaluated together. Median Ct values in samples obtained within the first five days of symptom onset were lower in patients with severe disease when compared to mild and moderate, but the difference was not statistically significant. Second and third week Ct values were also similar in the mild, moderate, and severe disease groups.

In the second week after symptom onset, SARS-CoV-2 RNA positivity continued in only 17% (n = 20) of the 117 patients who had a repeated RT-PCR test. In these patients, the highest positivity rate (10/44; 22.7%) was detected in the moderate group. The positivity was lower in the mild (9/64; 14.1%) and severe (1/9; 11.1%) disease groups, but the difference was not statistically significant. When 90 patients who had repeated RT-PCR tests, were evaluated in the third week, there was no statistical difference between the severity of COVID-19 disease and the continued PCR positivity.

According to their real-time RT-PCR Ct values, patients were divided into two groups: with a high (Ct value ≤ 25) and a low (Ct value > 25) viral load. Table 4 shows the comparisons in disease severity, age, sex, comorbidity, lung CT results, and baseline laboratory findings (1–3 days) in terms of viral load. The viral load was low (Ct value > 25.0) in 63% of the patients. Table 4 reveals that gender, age, presence of comorbidity, disease severity, lung CT findings, and mortality were similar between patients with high and low viral loads ($p > 0.05$).

In addition, inflammatory, biochemical and hematological data were evaluated. The count of thrombocytes, white blood cells, neutrophils, and lymphocytes were significantly lower in patients with a high viral load (Ct ≤ 25). Among biochemical and inflammatory parameters, only creatine kinase-myocardial band (CK-MB) value was lower ($p = 0.025$) in patients with low Ct values. The other biochemical and inflammatory parameters did not differ between the two groups (Table 4).

Discussion

COVID-19 covers a heterogeneous spectrum of disease courses. For this reason, the prediction of prognosis of patients at the time of diagnosis will greatly contribute to the treatment and patient management decisions. In general, the rate of severe cases reported is 4–16% [10,11]. This rate has been found 10.7% in the present study, which was conducted when the original Wuhan/D614G variant was circulating in Turkey.

It is reported that old age and the presence of comorbidities, such as hypertension, coronary artery disease, and malignancy are associated with poor prognosis in COVID-19 [12-15]. We found that severe infections were observed more frequently in patients

Table 3. Comparison of Ct values and positivity in patients with mild, moderate, or severe disease.

	Disease severity (n)				
	Total (214)	**Mild (105)**	**Moderate (86)**	**Severe (23)**	***p***
First week Ct values, median (IQR)					
1–5 days (n = 181)	26.5 (21.6–33.3)	27.6 (21.6–35.5)	25.8 (21.8–32.2)	26.0 (21.4–33.3)	0.200
6–7 days (n = 33)	33.4 (28.8–38.3)	35.8 (29.2–39.1)	32.0 (27.4–36.5)	32.3 (28.4–36.3)	0.200
Second week Ct values, median (IQR) (n = 117)	33.0 (28.5–34.9)	34.2 (25.3–35.2)	32.7 (27.9–34.9)	32.3 (31.1-33.5)	0.250
Third week Ct values, median (IQR) (n = 90)	36.7 (31.2–38.3)	34.9 (31.4–38.3)	36.7 (30.2–38.6)	–	0.230

IQR: interquartile range; Ct: cycle threshold.

with advanced age, similar to other studies [16,17]. Despite the fact that healthcare workers had a relatively high incidence of COVID-19 in this study, the number of severe diseases in this group was lower. Low number of severe disease cases may be due to the low average age of healthcare workers and the fact that they had fewer comorbid conditions as reported in another study [18]. A meta-analysis recently published by Fathi *et al*. showed that hypertension (28.3%), cardiovascular disease (19.7%), and diabetes (14.3%) were the most common comorbid diseases in COVID-19 patients [16]. In the present study, the three most common comorbid diseases were detected at similar rate as previous studies and were found associated with severe disease [5,12,15,16,19].

SARS CoV-2 viral dynamics has been investigated by researchers to understand the course of COVID-19 [20–22]. Viral loads of SARS-CoV-2 varies during the course of infection; the highest viral load values (lowest Ct values) have been reported within the first week of the disease, and the highest viral loads are detected soon after symptom onset [23–26]. The WHO has reported that higher viral load in upper respiratory tract samples that occurs between 0 and 4 days after symptom onset, decreases by half (54%) after 10–14 days, and the average virus detection time is 12 days [27]. In our study, the lowest Ct value was recorded on the third day after onset of symptoms, and the positivity rates decreased significantly during the second and third weeks (to 17.1% and 7.8%, respectively). A positive or

Table 4. Clinical and laboratory characteristics of 214 patients according to viral loads determined by real time RT-PCR Ct values.

	High viral load (Ct ≤ 25) n = 79 (36.9%)	**Low viral load (Ct > 25) n = 135 (63.1%)**	***p***
Disease Severity			
Mild	39 (49.4)	66 (48.9)	
Moderate	31 (39.3)	55 (40.7)	0.961
Severe	9 (11.4)	14 (10.4)	
Gender			
Male	42 (53.2)	73 (54.1)	0.898
Female	37 (46.8)	62 (45.9)	
Age Median (IQR)	41 (20.0)	42 (22)	0.745
≥ 65 (n = 19)	10 (12.7)	8 (5.6)	0.09
< 65 (n = 195)	69 (87.4)	126 (93.3)	
Comorbidity [n (%)]			
HT	10 (12.0)	20 (14.0)	0.661
DM	8 (10.1)	11 (8.1)	0.623
CVD	8 (10.1)	11 (8.1)	0.623
CPD	10 (12.7)	9 (6.7)	0.100
Immune compromise	3 (3.8)	7 (5.2)	0.748
Malignancy	2 (2.5)	6 (4.4)	0.700
Computerize tomography results* [n = 204 (%)]			
Bilateral pulmonary infiltrates	24 (30.8)	44 (34.1)	0.650
Focal/unilateral pulmonary infiltration	14 (17.9)	20 (15.5)	0.641
Atypical for COVID-19	2 (2.6)	6 (4.7)	0.713
Disease severity/support			
Invasive mechanical ventilation	3 (3.8)	6 (4.4)	0.690
Noninvasive O_2 support	7 (8.9)	10 (7.4)	0.635
Mortality (30^{th} day)	6 (7.6)	5 (3.7)	0.104
1–3 day laboratory characteristics; median (IQR)			
WBC	5.7 (4.7–7)	6.3 (5.6–9)	< 0.001
Neutrophil 10^3 K/μL	3.5 (2.7–4.5)	4.2 (3.0–5.8)	0.001
Lymphocyte 10^3 K/μL	1.1 (1.0–1.9)	1.4 (1.3–2.7)	< 0.001
Thrombocyte 10^3 K/μL	194.1 (169.2-261.8)	204.4 (180.1–275.7)	0.046
CRP mg/L	5.0 (2.0–14.7)	27.4 (2.0–32)	0.611
PCT %	0.2 (0.1–0.2)	0.2 (0.1–0.2)	0.284
Ferritin μg/L	76.0 (34–207)	156 (39–248.1)	0.120
D-Dimer mg/L	0.4 (0.2–0.6)	0.5 (0.3–0.7)	0.101
LDH U/L	209 (176.0–262.5)	234 (172.0–277)	0.556
CK IU/L	0 (54.0–102.5)	79 (53.0–100)	0.557
CK-MB IU/L	11 (9.0–14.8)	14 (10.0–17.8)	0.025
Oponin ng/L	1.7 (0.9–4.2)	3.3 (1.2– 4.4)	0.373

*In any period of the disease. IQR: interquartile range; HT: Hypertension; DM: Diabetes mellitus; CVD: Cardiovascular disease; CPD: Chronic pulmonary disease (Asthma and obstructive diseases); CRP: C-reactive protein; PCT: Prokalcitonin; LDH: Lactate Dehydrogenase; CK: Creatine kinase; CK-MB: Creatine kinase-myoglobin binding.

negative qualitative SARS-CoV-2 RT-PCR test result is sufficient for diagnosis; however, Ct values may be important in predicting the stage of the disease. Therefore, we would like to emphasize the importance of time of testing and onset of symptoms when comments on Ct values are expected to be evaluated. The time of symptoms onset can provide an estimate for the days with a higher viral load. It is also useful for the choice of diagnostic assays (e.g., antigen, PCR, or serological test) and detection of contagious periods. Prolonged viral shedding has been reported in some studies but in this study we have not followed the patients after 4 weeks [28, 29].

SARS-CoV-2 viral load may be affected by immunological parameters (e.g., viral neutralizing antibodies) besides viral dynamics (e.g., SARS-CoV-2 variants). Effective antibody response could be suspected in the first-week post-symptoms in about half of the patients. However, in the present study serological parameters have not been addressed. For baseline Ct values, 75% of the samples have been taken in the first three days of symptoms and sample collection was completed in the first week of disease (before antiviral treatment).

The median SARS-CoV-2 RT-PCR Ct value was 28.2 (IQR, 22.2–33.8) in the present study. This finding is in complete agreement with the median Ct value (28.16; IQR, 24.5–31.6) reported by Karahasan *et al*. using the same RT-PCR kit in our country [15]. In another study, the median Ct value was varying according to the target gene; for example, the median Ct value was different for *N*, *E* and *RdRP* genes in positive samples. [31]. Therefore, it should be noted that Ct values may vary when using different target genes in kits [20].

A recent review points out that there is a direct association of Ct values with the clinical outcomes of 15 studies (70%) that were evaluated [20]. However, some studies reported no statistically significant difference between asymptomatic and symptomatic patients with COVID-19 in terms of the viral load in nasal swabs determined via Ct values [20,26,33]. Our results share similarities with the other two studies (one with adults and one with children) conducted in our country that compared Ct values with disease severity. No difference was found between Ct values and factors such as gender, age, comorbidity, and disease severity [15,32]. A SARS-COV-2 real-time RT-PCR kit was rapidly manufactured at the beginning of the COVID-19 epidemic and was sent to all of the laboratories to be used to benefit the public health in our country. Thus, no serious problems arose for finding diagnostic PCR kits and their consumables and homogeneity were achieved in terms of the test results. Until now, besides our study, only two studies have compared Ct values with disease severity and course of disease in our country, and their results were similar.

Using Ct values as a proxy for viral load is influenced by the kit specifications as well as factors within the sample matrix that can affect amplification efficiency. Furthermore, the sampling conditions and the nasopharyngeal samples are not standardized. Therefore, we suggest that it may be misleading to comment on the severity of the disease based on the Ct values. The possibility of standardization and sensitivity problems in upper respiratory tract samples suggest that using lower respiratory tract samples may be advantageous for viral load quantification [34]. At the same time, standardized quantitative kits are more compliant for obtaining a convenient interpretation of the viral loads, and further studies are needed in this regard.

Hematological changes such as lymphopenia, thrombocytopenia, and leukopenia have been detected (5-41.7%) in COVID-19 patients. Thrombocytopenia may result from the consumption of platelets in damaged lungs and/or reduced production. Lymphopenia is clearly associated with disease severity and generally occurs along with leukopenia in COVID-19 infection. Nevertheless, in some patients, WBC count remains in the normal range [12,23,35]. Besides these data, Huang *et al.* found a lower count of lymphocytes, basophils and eosinophils, but higher neutrophil counts in hospitalized COVID-19 patients with high viral loads [12]. Despite the positive correlation between Ct values and lymphocyte counts, WBC counts may be normal or decreased in patients with low Ct values [12,35,36]. In this study, the basal (1–3 days) WBC, neutrophil, thrombocyte and lymphocyte counts were significantly lower in patients with low Ct values ($Ct \leq 25$) compared to those with high Ct values ($Ct > 25$). Although neutrophil and thrombocyte counts differ in the two groups (patients with low Ct values and patients with high Ct values), median values were within the normal range. Lymphocyte count was significantly lower in patients with low Ct values than in patients with high Ct values, and median lymphocyte count was also below normal values in the cohort. Huang *et al.* suggested that high SARS-CoV-2 viral loads affect lymphocyte counts resulting in lymphopenia [12]. In our opinion lower lymphocyte counts may cause and/or result in higher viral loads. The evidence from this study suggests that a reduction in lymphocyte count may have a direct

effect on viral load and there is a direct correlation between low lymphocyte ratios and low Ct values. New studies based on immunology would be useful in interpreting and explaining the underlying molecular dynamics of this finding.

Conclusions

SARS-CoV-2 RT-PCR Ct values in nasopharyngeal samples were lowest (viral load is highest) on the third day and increased significantly from the sixth day and later. Co-morbidity and age were risk factors for a severe disease course in COVID-19. No significant association was detected between the severity/mortality of the disease and Ct values on admission or at later stages of disease. The SARS-CoV-2 viral load has been evaluated by different qualitative RT-PCR tests and non-standardized samples so far. This may cause discordant results and it may be misleading to comment on the clinical outcome of COVID-19 based only on Ct values. However, low neutrophil, lymphocyte, and platelet counts may predict lower Ct values (namely high viral load).

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Authors' contributions

All authors contributed to the writing of the final manuscript and approved its publication. Contributors IS, BE, and HA were responsible for the organization and coordination of the study. IS was the chief investigator and GO performed the statistical analyses. BO, BY, NAAO and AU collected and interpreted the data. Authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy of any part of the work are appropriately investigated and resolved.

References

1. World Health Organization (WHO) (2019) Coronavirus disease (COVID-19) weekly epidemiological update and weekly operational update. Available:https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed: 10 May 2021.
2. Bohn MK, Hall A, Sepiashvili L, Jung B, Steele S, Adeli K (2020) Pathophysiology of COVID-19: mechanisms underlying disease severity and progression. Physiology (Bethesda) 35: 288-301.
3. Lai CC, Ko WC, Lee PI, Jean SS, Hsueh PR (2020) Extra-respiratory manifestations of COVID-19. Int J Antimicrob Agents 56: 106024.
4. Kuderer NM, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR (2020) Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. Lancet 395: 1907-1918.
5. Ji W, Huh K, Kang M, Hong J, Bae GH, Lee R, Na Y, Choi H, Gong SY, Choi YH, Ko KP, Im JS, Jung J (2020) Effect of underlying comorbidities on the infection and severity of COVID-19 in Korea: a nationwide case-control study. J Korean Med Sci 35: e237.
6. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall S, Manson JJ, HLH Across Speciality Collaboration, UK (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 395: 1033–1034.
7. 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 (2020) Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. Clin Infect Dis 71: 2663-2666.
8. Ade C, Pum J, Abele I, Raggub L, Bockmühl D, Zöllner B (2021) Analysis of cycle threshold values in SARS-CoV-2-PCR in a long-term study. J Clin Virol 138: 104791.
9. World Health Organization (WHO) (2020) COVID-19 Clinical management: living guidance. Available: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1. Accessed: 10 May 2021.
10. Hu B, Guo H, Zhou P, Shi ZL (2021) Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 19: 141-154.
11. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19 (2020) Clinical characteristics of Coronavirus Disease 2019 in China. New Engl J Med 382: 1708-1720.
12. Huang JT, Ran RX, Lv ZH, Feng LN, Ran CY, Tong YQ, Li D, Su HW, Zhu CL, Qiu SL, Yang J, Xiao MY, Liu MJ, Yang YT, Liu SM, Li Y (2020) Chronological changes of viral shedding in adult inpatients with COVID-19 in Wuhan, China. Clin Infect Dis 71: 2158-2166.
13. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W (2020) Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis 20: 656-657.
14. Yan Y, Yang Y, Wang F, Ren H, Zhang S, Shi X, Yu X, Dong K (2020) Clinical characteristics and outcomes of patients with severe covid-19 with diabetes. BMJ Open Diabetes Res Care 8: e001343.
15. Karahasan Yagci A, Sarinoglu RC, Bilgin H, Yanılmaz Ö, Sayın E, Deniz G, Guncu MM, Doyuk Z, Barıs C, Kuzan BN, Aslan B, Korten V, Cimsit C (2020) Relationship of the cycle threshold values of SARS-CoV-2 polymerase chain reaction

and total severity score of computerized tomography in patients with COVID 19. Int. J Infect Dis 101: 160–166.
16. Fathi M, Vakili K, Sayehmiri F, Mohamadkhani A, Hajiesmaeili M, Rezaei-Tavirani M, Eilami O (2021) The prognostic value of comorbidity for the severity of COVID-19: a systematic review and meta-analysis study. PLoS One 16: e0246190.
17. Centers for Disease Control (CDC) (2019) Deaths involving coronavirus disease 2019 (COVID-19) by race and Hispanic origin group and age, by state. Available: https://data.cdc.gov/NCHS/Deaths-involving-coronavirus-disease-2019-COVID-19/ks3g-spdg. Accessed: 20 June 2021.
18. Gómez-Ochoa SA, Franco OH, Rojas LZ, Raguindin PF, Roa-Díaz ZM, Wyssmann BM, Guevara SLR, Echeverría LE, Glisic M, Muka T (2021) COVID-19 in health-care workers: a living systematic review and meta-analysis of prevalence, risk factors, clinical characteristics, and outcomes. Am J Epidemiol 190:161-175.
19. Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, Xu W, Zhang C, Yu J, Jiang B, Cao H, Li L (2020) Clinical characteristics of imported cases of COVID-19 in Jiangsu Province: a multicenter descriptive study. Clin Infect Dis. 71: 706–712.
20. Rao SN, Manissero D, Steele VR, Pareja J (2020) A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther 9: 573–586.
21. La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, Gautret P, Raoult D (2020) Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis 39: 1059–1061.
22. 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 (2020) Predicting infectious Severe Acute Respiratory Syndrome Coronavirus 2 from diagnostic samples. Clin Infect Dis 71: 2663-2666.
23. Liu Y, Liao W, Wan L, Xiang T, Zhang W (2021) Correlation between relative nasopharyngeal virus RNA load and lymphocyte count disease severity in patients with COVID-19. Viral Immunol 34: 330-335.
24. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q (2020) Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 20: 411-412.
25. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J (2020) SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 382: 1177-1179.
26. 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 (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26: 672–675.
27. World Health Organization. (WHO) (2020) Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected. Available: https://www.who.int/publications-detail/clinicalmanagement-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed: 25 May 2021.
28. Li TZ, Cao ZH, Chen Y, Cai MT, Zhang LY, Xu H, Zhang JY, Ma CH, Liu Y, Gao LJ, Duan ZH, Mou DL, Liang LC (2021) Duration of SARS-CoV-2 RNA shedding and factors associated with prolonged viral shedding in patients with COVID-19. J Med Virol 93: 506–512.
29. Xiao AT, Tong YX, Zhang S (2020) False negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: rather than recurrence. J Med Virol 92: 1755-1756.
30. Kleiboeker S, Cowden S, Grantham J, Nutt J, Tyler A, Berg A, Altrich M (2020) SARS-CoV-2 viral load assessment in respiratory samples. J Clin Virol 129: 104439.
31. Drew RJ, O'Donnell S, LeBlanc D, McMahon M, Natin D (2020) The importance of cycle threshold values in interpreting molecular tests for SARS-CoV-2. Diagn Microbiol Infect Dis 98: 115130.
32. Aykac K, Cura Yayla BC, Ozsurekci Y, Evren K, Oygar PD, Gurlevik SL, Coskun T, Tasci O, Demirel Kaya F, Fidanci I, Tasar MA, Alp A, Cengiz AB, Karahan S, Ceyhan M (2021) The association of viral load and disease severity in children with COVID-19. J Med Virol 9: 3077-3083.
33. Kimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, Bardossy AC, Oakley LP, Tanwar S, Chisty Z, Bell JM, Methner M, Harney J, Jacobs JR, Carlson CM, McLaughlin HP, Stone N, Clark S, Brostrom-Smith C, Page LC, Kay M, Lewis J, Russell D, Hiatt B, Gant J, Duchin JS, Clark TA, Honein MA, Reddy SC, Jernigan JA; Public Health – Seattle & King County; CDC COVID-19 Investigation Team (2020) Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility - King County, Washington, March 2020.MMWR Morb Mortal Wkly Rep. 69: 377–381.
34. Yu X, Sun S, Shi Y, Wang H, Zhao R, Sheng J (2020) SARS-CoV-2 viral load in sputum correlates with risk of COVID-19 progression. Crit Care 24:170.
35. Liu X, Zhang R, He G (2020) Hematological findings in coronavirus disease 2019: indications of progression of disease. Ann Hematol 99: 1421-1428.
36. Yuan C, Zhu H, Yang Y, Cai X, Xiang F, Wu H, Yao C, Xiang Y, Xiao H (2020) Viral loads in throat and anal swabs in children infected with SARS-CoV-2. Emerge Microbes Infect 9: 1233–1237.

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